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**VOCAL DUETTING BEHAVIOUR IN A NEOTROPICAL WREN: INSIGHTS INTO PATERNITY
GUARDING AND PARENTAL COMMITMENT**

By

ZACHARY ALEXANDER KAHN

A Thesis

Submitted to the Faculty of Graduate Studies
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
at the University of Windsor

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2017

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Vocal duetting behaviour in a neotropical wren: Insights into paternity guarding and parental commitment

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Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

I am the sole author of chapters 1 and 4, and the principal author of chapter 2 and 3. Both chapter 2 and 3 were conducted under the supervision of Dan Mennill (University of Windsor), who contributed valuable input on the experimental design, analyses, and writing of the manuscripts, as well as logistical and financial support for field research in Costa Rica and lab research in Windsor; he shares authorship on both chapters 2 and 3. Chapter 2 of the thesis was co-authored by Chris Moser-Purdy (University of Windsor), who helped with field research and contributed valuable input to the writing of the manuscript. Chapter 3 of the thesis was co-authored by Sarah Tremain-Douglas and Kristin Kovach (University of Windsor), who contributed significantly with data collection and also helped with the writing of the manuscript. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation were performed by myself, with input from my co-authors.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis.

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Abstract

In many tropical animals, male and female breeding partners combine their vocalizations to produce elaborate vocal duets. Although duets are produced by diverse animal taxa, the functions of these coordinated vocalizations remain poorly understood. In this thesis, I explored the ecology and evolution of vocal duetting behaviour by testing two poorly-studied hypotheses for duet function in a Neotropical duetting songbird, the Rufous-and-white Wren (*Thryophilus rufalbus*). The Paternity Guarding Hypothesis states that male animals create duets with their females to guard against other males seeking mating opportunities. I used a playback experiment to test this hypothesis by first simulating an intrusion from a rival male, and then simulating a subject males breeding partner to give him opportunities to create duets with his female during both the fertile and non-fertile periods. Consistent with predictions of the Paternity Guarding Hypothesis, males created more duets with their partners during the fertile period compared to the non-fertile period, suggesting that they used duets to acoustically protect their parentage. The Signalling Commitment Hypothesis states that singing duets with a partner signals willingness or ability to invest effort into a monogamous partnership. I tested this hypothesis by investigating the relationship between duetting behaviour and future parental investment. I found no evidence of a positive relationship between male or female duetting behaviour and future investment in nest-building or nestling-provisioning, and therefore my data provide no support for the Signalling Commitment Hypothesis. My research provides new insight into the evolution and functions of vocal duets in tropical animals, revealing that duets play an important role in paternity guarding, but do not signal future parental commitment in Rufous-and-white Wrens.

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Chapter 1: General Introduction

Introduction

Animals use acoustic signals to serve many different communication functions, including territory acquisition and defense, predator detection and avoidance, mate attraction and mate guarding, and communication between breeding partners (Bradbury and Vehrencamp 2011). Acoustic signals have several properties that make them ideal for communication, such as their long range, ability to transmit around physical obstructions, and the ability of animals to modulate the amplitude of these signals for different receivers (Bradbury and Vehrencamp 2011). Acoustic communication is especially prevalent in birds, which are well-known for the variety and complexity of their vocalizations. The most well-studied acoustic signals in birds are their songs, which have been traditionally defined as long and complex vocalizations produced by males during the breeding season (Catchpole and Slater 2008). The functions of bird songs have been studied primarily in the Temperate Zone, where males sing to defend territories and attract females during the breeding season (Krebs 1977; Eriksson and Wallin 1986; Catchpole and Slater 2008). However, in tropical regions, where species diversity and richness is much higher, song is often produced by both sexes (Stutchbury and Morton 2001). Despite this, the functions of female song have received less empirical attention (Langmore 1998; Slater and Mann 2004). Interestingly, female song is much more widespread than previously recognized (it occurs in 71% of all songbird species and in a diverse group of avian families) and female song is an ancestral trait in songbirds (Odom et al. 2014).

In many animals, particularly in the tropics, male and female breeding partners combine their vocalizations to produce coordinated vocal duets. Vocal duets, which occur when an individual answers its partner's vocalization by overlapping it or vocalizing shortly afterwards,

are considered to be one of the most complex acoustic signals in the animal kingdom (Mann et al. 2003; Hall 2004). Duetting behaviour occurs in a wide range of animal taxa, including primates (Kinzey and Robinson 1983; Geissman 2002; Caselli et al. 2015), anurans (Tobias et al. 1998; Emerson and Boyd 1999), cetaceans (Lilly and Miller 1961), insects (Bailey 2003), bats (Carter et al. 2008), and many species of birds (Farabaugh 1982; Hall 2004; Tobias et al. 2016). Duetting has received considerable attention in birds; recent studies have shown duetting occurs in 49% of avian families, and in as many as 16% of all species (Tobias et al. 2016). The functions of avian duets, however, remain poorly understood, largely because of a historical research focus in temperate areas where duetting is less common (Langmore 1998; Slater and Mann 2004). In this Master's thesis, I explore the ecology and evolution of vocal duetting behaviour in tropical animals by investigating two rarely-studied hypotheses for duet function in novel ways. In this General Introduction, I provide a broad overview of the topics that are relevant to this thesis, to set the stage for the two data chapters that follow.

The function of vocal duets

The most widely-supported function of avian vocal duets is joint territory defense, whereby pairs of birds produce duets as cooperative signals to defend ecological resources from rival pairs (reviewed in Dahlin and Benedict 2014). Many duetting species form long-term pair bonds, hold territories year-round, experience prolonged breeding seasons, and exhibit convergent sex roles with respect to breeding and territory defense, suggesting that there is increased selection in these species to defend ecological resources over longer time frames (Benedict 2008; Logue and Hall 2014; Tobias et al. 2016). The role of duets in territory defense has been demonstrated experimentally in many species (Douglas and Mennill 2010; Dahlin and

Benedict 2014). For example, mated pairs of Yellow-naped Amazon Parrots (*Amazona auropalliata*) produce duets at equal rates in response to male, female, and paired intruders, suggesting that duets are aggressive signals used to defend territories from multiple types of intruders (Dahlin and Wright 2012). Similarly, Barred Antshrikes (*Thamnophilus doliatus*) sing duets at similar rates in response to male, female, and paired intruders, and sing more duets in response to dual-speaker duet playback compared to duets from a single speaker, indicating that duets are threatening signals used to defend ecological resources from conspecific animals (Koloff and Mennill 2011, 2013).

In addition to the role they play in cooperative territory defense, duets have also been proposed to function in other cooperative contexts, such as coordinating of reproductive activities (e.g. Topp and Mennill 2008; Benedict 2010), maintaining contact in dense habitats (e.g. Logue 2007; Mennill and Vehrencamp 2008), and signalling pair bond strength (e.g. Hall and Magrath 2007; Rivera-Cáceras et al. 2016). Although duets clearly serve some cooperative functions that benefit both breeding partners, duets are also associated with intersexual conflict between breeding partners. In particular, an animal may create duets with their partner to prevent their partner from mating or pairing with same-sex rivals (Sonnenschein and Reyer 1983; Levin 1996a,b; Seddon and Tobias 2006). For example, female Eastern Whipbirds (*Psophodes olivaceus*) answer a higher proportion of male songs to create duets in response to same-sex playback compared to opposite-sex and paired playback, suggesting that duets are used to guard males from rival females (Rogers et al. 2007). Understanding the relative importance of cooperation and conflict in driving duetting behaviour is an important theme in this field of research.

Extra-pair paternity and paternity guarding

Although most birds are socially monogamous, most species exhibit a mixed reproductive strategy, where animals copulate with individuals other than their social mate (reviewed in Griffith 2002). Given the high fitness cost of these extra-pair copulations, many species have evolved behaviours to prevent their partners from engaging in a mixed reproductive strategy, including frequent copulations during the fertile period (reviewed in Møller and Birkhead 1991), courtship displays as a bribing mechanism (Green and Krebs 1995; Velando 2004), physical mate guarding (reviewed in Birkhead and Møller 1992), and increased song rates during the fertile period (i.e. acoustic mate guarding; Sexton et al. 2007; Bruni and Foote 2014).

Although extra-pair paternity and paternity-guarding strategies have been well-studied in many temperate bird species, far less is known about the genetic mating systems and associated paternity guarding behaviours of tropical species, particularly in duetting species (Macedo et al. 2008; Douglas et al. 2012). Interestingly, mixed reproductive strategies appear to vary between non-duetting versus duetting species; current evidence suggests that rates of extra-pair paternity across duetting species are low (reviewed in Douglas et al. 2012), although the dataset available for comparative study is small. One possible explanation for this pattern is that duets may be used by males as acoustic paternity guards, an idea that has come to be known as the Paternity Guarding Hypothesis (Hall 2004). According to the Paternity Guarding Hypothesis, male birds answer their partner's songs to create duets in order to advertise their partner's mated status and thereby minimize extra-pair copulations between their partner and same-sex rivals (Sonnenschein and Reyer 1983; Hall 2004). Two main predictions arise from this

hypothesis: (1) male birds should participate in more duets with their female when she is fertile, because this is when they are most at risk of losing paternity to rivals male; and (2) male birds that create more duets with their females should be less likely to lose parentage to extra-pair sires.

Current evidence for the Paternity Guarding Hypothesis for vocal duets is equivocal. In Buff-breasted Wrens (*Cantorchilus leucotis*), Magpie-larks (*Grallina cyanoleuca*), Purple-crowned Fairy-wrens (*Malurus coronatus*), and Red-backed Fairy-wrens (*Malurus melanocephalus*), males do not perform more duets in the fertile period compared to other breeding periods, although the reverse is true in male Rufous-and-white Wrens (*Thryophilus rufalbus*; Hall 2000; Gill et al. 2005; Topp and Mennill 2008; Hall and Peters 2009; Dowling and Webster 2013). Several other studies have used a more direct approach to test this hypothesis, by investigating how duetting behaviour relates to rates of extra-pair offspring in nests. In Crimson-breasted Shrikes (*Laniarius atrococcineus*), males that sing more duets with their females do not suffer reduced rates of extra-pair paternity in their nests, but Red-backed Fairy-wren males that create more duets with their females during a simulated intrusion suffer fewer extra-pair offspring in their nests (Van den Heuvel et al. 2014; Baldassare et al. 2016). Despite this, few studies to date have experimentally tested the Paternity Guarding Hypothesis by simulating an aggressive context and assessing how male duetting behaviour changes with changes in female fertility status.

Signalling partnership commitment

Many animals can evaluate the quality of potential breeding partners by assessing behavioural and ornamental traits, including colouration (e.g. Hill 1991; Smith et al. 2014), acoustic signals (e.g. Buchanan and Catchpole 2000; Forsman and Hagman 2006), physical ornaments (e.g. Voltura et al. 2002; Tibbetts et al. 2015), and courtship displays (e.g. Knapp and Kovach 1991; Green and Krebs 1995). Animals may use these traits to assess the benefits that they will receive by breeding with the individual possessing the trait, including both indirect benefits (e.g. higher quality genes; Hamilton and Zuk 1982) and direct benefits (e.g. access to better ecological resources or parental care; Andersson 1994). However, these traits have been primarily studied in the context of sexual selection in temperate species, whereby female animals assess male traits prior to mate selection. In contrast, in many tropical animals, breeding partners form long-term pair bonds, experience prolonged breeding seasons, and defend territories year round, suggesting that breeding partners may need to assess phenotypic traits of their mates outside of the context of mate selection (reviewed in Wachtmeister 2001). Surprisingly few studies have investigated the importance of ornamental traits after pairing (i.e. post-pairing displays), despite their prevalence in a variety of taxa and potential importance in mate assessment and strengthening long-term pair bonds (Wachtmeister 2001).

Many socially monogamous bird species produce vocal duets as post-pairing displays that continue to occur after pair formation and for large portions of the year (e.g. Topp and Mennill 2008; Benedict 2010). One rarely-studied hypothesis for the function of duets is that duets signal commitment to a partnership, where commitment represents willingness or ability to invest effort into future reproduction, territory defense, predator vigilance, or other

components of the partnership (Hall 2004). According to the Signalling Commitment Hypothesis, components of duetting behaviour, such as answering a partner's song to create a duet, should provide an honest indication about the quality of the partner and the willingness or ability to invest effort in future reproduction (Wickler 1980; Hall 2004). Many studies have shown a positive association between acoustic traits in birds, such as song rate (e.g. Greig-Smith 1982) and song complexity (e.g. Buchanan and Catchpole 2000), and future parental investment (e.g. increased nestling provisioning rates). However, few studies have tested the Signalling Commitment Hypothesis in duetting species by investigating how duetting behaviour relates to future parental investment, and therefore whether duets function as post-pairing displays for mate assessment or signalling parental commitment.

Study species and location

In this thesis, I explore vocal duetting behaviour by investigating two hypotheses for duet function: the Paternity Guarding Hypothesis and the Signalling Commitment Hypothesis. I conducted my research in a long-term study population of Rufous-and-white Wrens (*Thryophilus rufalbus*), a Neotropical songbird with a distribution from southern Mexico, throughout much of western Central America, and into parts of northern Colombia and northwestern Venezuela (Stiles and Skutch 1989; Mann et al. 2009). Rufous-and-white Wrens are medium-sized passerines which are sexually monochromatic but exhibit slight sexual dimorphism, with males being slightly larger than females (males: 25.8 ± 0.4 g; females: 23.7 ± 0.5 ; Mennill and Vehrencamp 2005). Adults exhibit a prominent rufous colour on the head, mantle, wings, and tail, with white on the throat and underparts. Rufous-and-white Wrens are relatively long-lived songbirds that defend territories year-round and form long-term breeding

partnerships. My study population is located in Sector Santa Rosa of the Area de Conservación Guanacaste (10°51'N, 85°36'W), a tropical dry forest in which resident wrens inhabit mature humid and late-successional regrowth forests. Dan Mennill and his students at the University of Windsor have been studying the vocal behaviour and ecology of this population since 2003 as part of a long-term monitoring project; birds are colour-banded and recorded each year.

Male and female Rufous-and-white Wrens both sing using a repertoire of songs (males have an average repertoire size of 11 songs; females 9 songs; Mennill and Vehrencamp 2005). Their songs are flute-like and tonal, with introductory, middle, and terminal syllables, sung at relatively low frequencies to transmit effectively through dense vegetation (Mennill and Vehrencamp 2005). Breeding partners use their songs as solos, or combine them as vocal duets. Duets involve one bird singing a song, and their partner – the second bird to sing – producing a song ≤ 1.0 seconds afterwards to create the duet. Both males and females can create duets in response to their partner's songs, but the majority of duets overall are created by females (females create 73% of all duets; Topp and Mennill 2008). There is considerable variation in how the sexes use their songs throughout the year. During the dry pre-breeding season, females sing at higher rates and answer a higher proportion of male songs to create duets, while males exhibit the opposite pattern (Topp and Mennill 2008). During the rainy breeding season, males sing songs at higher rates and answer a higher proportion of female songs to create duets, while females reduce their solo song and duetting rates (Topp and Mennill 2008).

Rufous-and-white Wrens begin to breed each year at the beginning of the rainy season, which typically occurs at the beginning of May and continues until August (Topp and Mennill 2008). Nest-building begins at the end of the dry season, immediately after the first large

rainfall of the year. Both males and females contribute to building breeding nests, which are large globular structures comprised of grass and black fungal rhizomes with a downward-facing entrance tube (Stiles and Skutch 1989). Pairs primarily build nests in bullhorn acacia trees (*Vachellia collinsii*) 2-10 m off the ground. Males also build secondary nests that are used for subsequent breeding attempts. Females begin to lay eggs once nests are completed and the rainy season has begun, laying one egg each day until the clutch is complete (3-5 eggs is typical). Females incubate the eggs and brood the young (Topp and Mennill 2008). The incubation period lasts for 16-18 days (D. Mennill pers. obs.). Both males and females help to provision nestlings, but females contribute significantly more provisioning effort than males (males: 32% of trips; females: 68% of trips). Nest predation rates are high at our study site (approximately 80% of nests), and pairs will build new nests and attempt to breed several more times after their nests fail. Rufous-and-white Wrens follow a mixed reproductive strategy, with low rates of extra-pair paternity across the population (3% of offspring in 6% of broods), and all extra-pair sires coming from neighbouring males (Douglas et al. 2012).

Rufous-and-white Wrens duets are multi-purpose signals that have several different functions. Similar to other duetting species, the most well-supported function of duets is joint territory defense (Mennill 2006; Mennill and Vehrencamp 2008). For instance, Rufous-and-white Wrens respond to simulated duetting intruders by increasing their duetting rates (Mennill 2006). Similarly, birds sing duets at similar rates in response to same-sex, opposite-sex, and paired intruders, indicating that pairs sing duets to mutually defend territories from all types of intruders (Mennill and Vehrencamp 2008). Rufous-and-white Wrens also use duets to maintain contact with each other in dense habitats, given that birds often move towards each other after

singing duets (Mennill and Vehrencamp 2008). Finally, duets appear to serve important functions related to communication during breeding activities, given that birds sing duets with each at higher rates around their nest compared to others areas in their territory during the breeding season (Mennill and Vehrencamp 2008; Kovach 2013). Despite what is known about duet function in this species, it is less clear if duets serve any additional functions, particularly in conflict-based contexts. For example, despite the fact that males answer the highest proportion of female songs to create duets during the female fertile period, suggesting that these signals could serve a paternity guarding function, no study has directly tested the Paternity Guarding Hypothesis in this species (Topp and Mennill 2008). Similarly, no study in this species has investigated whether duets signal partnership commitment by indicating willingness or ability to invest effort into partnership aspects, such as parental investment.

Thesis goals

In this thesis, my goal is to explore the ecology and functions of vocal duetting behaviour by investigating two poorly-studied hypotheses for duet function in Rufous-and-white Wrens. In the first data chapter – Chapter 2 – my goal is to test the Paternity Guarding Hypothesis by experimentally investigating how male duetting behaviour is affected by female fertility status, and therefore whether or not duets function as acoustic paternity guards in this species. In the second data chapter – Chapter 3 – my goal is to test the Signalling Commitment Hypothesis by investigating the relationship between duetting behaviour and future parental investment, and whether duets can signal willingness to invest effort into a monogamous partnership. Together, these two chapters will expand our understanding of vocal duetting

behaviour across animals by testing two relatively poorly-studied hypotheses for duet function in novel ways.

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Chapter 2: Male Rufous-and-white wrens use duets and physical behaviours to guard their breeding partners

Chapter summary

In diverse animal taxa, particularly in tropical environments, breeding partners coordinate their vocalizations to produce vocal duets. The study of the function of these synchronized vocalizations is an active area of investigation, with empirical evidence supporting multiple hypotheses for duet function. One poorly-studied hypothesis is the Paternity Guarding Hypothesis, which states that male animals create duets with their partners to advertise their mated status and thereby minimize mating attempts by rival males. Evidence for this hypothesis is equivocal, although few studies have tested it with an experimental approach. Here we experimentally test the Paternity Guarding Hypothesis in a colour-banded population of Rufous-and-white Wrens (*Thryophilus rufalbus*), a neotropical duetting songbird. We designed a two-part playback experiment, where males first experienced a simulated intrusion by a rival male, and were then given opportunities to answer their female partner's songs to create duets. We repeated this experiment during the female's fertile and non-fertile breeding stages. In support of predictions of the Paternity Guarding Hypothesis, male wrens created more duets with their partner during the fertile period compared to the non-fertile period. Additionally, male wrens appeared to physically guard their mates with greater intensity during the fertile period. Male song rates showed no significant variation between fertile and non-fertile periods, demonstrating that increased duetting in the fertile period was a result of a change in song use, rather than a change in song rate. Our study is among the first to experimentally test the Paternity Guarding Hypothesis for duet function, and suggests that male Rufous-and-white Wrens use both vocal and physical behaviours to guard their paternity.

Introduction

Tropical and temperate animals exhibit many differences in behavioural traits, including dramatic differences in their acoustic signalling behaviours (Morton 1996; Langmore 1998; Stutchbury and Morton 2001; Slater and Mann 2004). In temperate animals, elaborate vocalizations are primarily given by males, whereas in tropical animals, females are much more vocal, and there are diverse species where breeding partners combine their vocalizations into vocal duets (Langmore 1998; Hall 2004; Logue and Hall 2014; Tobias et al. 2016). Vocal duets are highly coordinated acoustic signals produced by mated pairs where one individual vocalizes and the partner creates a duet by vocalizing in response, either by overlapping the partner's song or producing a song in quick succession (Hall 2004). This behaviour occurs in diverse animal taxa, including primates (Geissman 2002; Caselli et al. 2015), insects (Bailey 2003), anurans (Tobias et al. 1998; Emerson and Boyd 1999), bats (Carter et al. 2008), cetaceans (Lilly and Miller 1961), and many species of birds (Hall 2004; Tobias et al. 2016).

Vocal duets are widely viewed to be multi-purpose signals that function in both cooperative and conflict-based contexts (Sonnenschein and Reyer 1983; Hall 2004; Marshall-Ball et al. 2006; Rogers et al. 2007; Mennill and Vehrencamp 2008; Benedict 2010; Dahlin and Benedict 2014). The most well-supported function for vocal duets is that they are used to cooperatively defend ecological resources (reviewed in Douglas and Mennill 2010 and Dahlin and Benedict 2014). However, a contrasting theory is that duets can arise from intersexual conflict between breeding partners; an animal may perform a duet with their partner to prevent their partner from pairing or mating with other animals (Sonnenschein and Reyer 1983; Levin 1996; Seddon and Tobias 2006; Rogers et al. 2007). A leading hypothesis under this view

is the Paternity Guarding Hypothesis, which states that males answer female vocalizations to create duets in order to advertise their partner's mated status and prevent their partner from mating with rival males (Sonnenschein and Reyer 1983; Hall 2004). Two important predictions arise from this hypothesis: (1) males should answer a higher proportion of female vocalizations to create duets during the fertile period in comparison to other breeding stages; and (2) duets should effectively reduce rates of extra-pair paternity (Hall 2004). Males in many well-studied temperate species have been shown to employ diverse paternity guarding strategies to reduce extra-pair paternity, including increased singing rates during the fertile period (e.g. Møller 1988; Sexton et al. 2007; Bruni and Foote 2014), frequent copulations (reviewed in Møller and Birkhead 1991), and physical mate guarding during the fertile period (reviewed in Birkhead and Møller 1992; Foote et al. 2008). However, few studies have investigated paternity guarding strategies in duetting species, particularly with respect to how duets may function as acoustic paternity guards.

Current evidence for the Paternity Guarding Hypothesis in duetting species has been equivocal. Most studies have involved observational analysis of variation in duetting rates across different breeding stages. For example, in Magpie-Larks (*Grallina cyanoleuca*), Buff-breasted Wrens (*Cantorchilus leucotis*), Purple-crowned Fairy-wrens (*Malarus coronatus*), and Red-backed Fairy-wrens (*Malarus melanocephalus*), males do not create more duets with their females during the fertile period compared to non-fertile periods (Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2009; Dowling and Webster 2013), suggesting that duets do not function as acoustic paternity guards in these species. The Paternity Guarding Hypothesis has only been tested experimentally in two duetting species. In response to playback simulating the

songs of a rival male during the female fertile period, male Canebrake Wrens (*Cantorchilus zeledoni*) produced more duet-initiation songs, suggesting that males are motivated to perform duets with females when they are fertile (Marshall-ball et al. 2006). In contrast, in response to playback simulating solo and paired intruders, male Red-backed Fairy-wrens (*Malurus melanocephalus*) did not sing more duets with their females in the fertile period compared to pre-fertile and-post fertile breeding stages (Dowling and Webster 2016), although, in another playback experiment, male Fairy-wrens that sang more duets with their females had lower reproductive losses to extra-pair paternity in their nests (Baldassere et al. 2016). In light of these conflicting results, more playback experiments are needed to empirically test the Paternity Guarding Hypothesis in duetting species.

In this study, we test the Paternity Guarding Hypothesis by conducting a playback experiment in a Neotropical duetting songbird: the Rufous-and-white Wren (*Thryophilus rufalbus*). Rufous-and-white Wrens provide an ideal species for testing the Paternity Guarding Hypothesis for two reasons. First, in the absence of playback, males answer the highest proportion of female songs to form duets during their fertile period compared to other breeding stages (Topp and Mennill 2008), suggesting that duets may serve a paternity guarding function. Second, Rufous-and-white Wrens in our study population exhibit low levels of extra-pair paternity (3% of offspring in 6% of broods, Douglas et al. 2012), indicating that duets may be effective paternity guards. We used playback to simulate a rival male near the edge of a subject's territory, creating the impression that a male competitor had entered the subject male's territory. We then used playback to simulate the subject's breeding partner near the centre of the subject's territory, thereby giving males an opportunity to create duets with their

partner's songs. We then assessed how subject male vocal and physical behaviour changed across fertile and non-fertile breeding stages. We made *a priori* predictions about the males' responses to playback based on the Paternity Guarding Hypothesis. After experiencing a simulated intrusion of a rival male, we predicted that males would answer a higher proportion of their female's songs to form duets (i.e. create more duets) in the fertile period compared to the non-fertile period. We also predicted that males would attempt to guard their paternity in two additional ways. We predicted that males would increase their independent song rate (i.e. songs that were not part of a duet), as has been observed in other studies of temperate songbirds (e.g. Møller 1988; Sexton et al. 2007). We also predicted that males would exhibit a more intense physical response towards the loudspeaker simulating their breeding partner (i.e. they would physically guard the loudspeaker simulating their breeding partner by approaching more closely, more quickly, and remaining near to the loudspeaker for longer) during the fertile period compared to the non-fertile period.

Methods

Study species and general field methods

We conducted our experiment in 2016 and 2017 in a colour-banded population of Rufous-and-white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica (10°51'N, 85°36'W). Rufous-and-white Wrens are Neotropical duetting songbirds found throughout many parts of Central and northwestern South America. Members of our laboratory group have studied this population since 2003, banding birds, mapping territories, finding nests, and recording songs on an annual basis. We banded all of the male

playback subjects in this experiment with unique combinations of coloured leg bands to facilitate individual identification, and we also banded 11 out of 21 of females (52%). We were confident in our ability to differentiate between the unbanded females based on their territory position and unique vocal repertoires (see Mennill and Vehrencamp 2005).

Playback experiment

We conducted playback experiments to 21 male Rufous-and-white Wrens between early May and mid-June across the two years of our study (7 pairs in 2016 and 14 pairs in 2017). This time of year coincides with the end of the non-breeding season and the beginning of the breeding season for Rufous-and-white Wrens (i.e. nest building and egg laying), which coincides with the first large rainfall of the year (Topp and Mennill 2008). Each male subject received playback on two occasions at different breeding stages. First, we delivered playback during the subject's partner's fertile period, which we defined as the window from five days before to two days after females laid their first egg of a clutch (Birkhead 1998). This period usually occurred during the first breeding attempt after the first large rainfall of the year ($n = 19$ subjects), or following subsequent failed breeding attempts due to nest predation ($n = 2$ subjects). Second, we delivered playback during the incubation period, which we defined as the period when females were incubating their completed clutch (females usually lay between 2-5 eggs and incubate for 12-15 days). We determined the breeding stage of each pair prior to conducting playback by finding and monitoring their nests, and by carefully observing the behaviour of birds during morning focal recordings.

Each playback experiment, both in the fertile and non-fertile period, included two stages of playback: the “Intrusion Stage” and the “Duetting Stage” (Figure 2.1). During the Intrusion Stage, we simulated a neighbouring male encroaching onto the subject’s territory, potentially seeking extra-pair copulations. We chose to simulate a neighbouring male, rather than a more distant male, because a previous genetic study of the mating system of Rufous-and-white Wrens found that all extra-pair sires were territorial neighbours, suggesting that neighbours are the most potent threat to a male’s paternity (Douglas et al. 2012). The Intrusion Stage was a “priming stage” that established a context of reproductive competition for the territorial male subject. We placed the Intrusion Stage loudspeaker near the shared territory boundary between the subject’s and neighbour’s territory.

During the second stage of playback, the Duetting Stage, we simulated the songs of the resident female using a loudspeaker placed near the centre of the subject’s territory, providing the subject male with an opportunity to perform duets with his breeding partner. (A similar protocol has proven successful in three other species of duetting wrens; Logue et al. 2008; Templeton et al. 2013; Rivera-Cáceras et al. 2016.) Female song output is typically quite low in our study species, and highly variable (Mennill and Vehrencamp 2008), and therefore we could not rely on the resident female to produce enough songs to test male responsiveness during the Duetting Stage. During the Duetting Stage, the subject had the opportunity to respond to his partners’ songs broadcast through the loudspeaker (30 songs in total) as well as any songs sung by his partner (these were rare; females produced an average of 5.0 ± 1.2 songs during the Duetting Stage). Although we considered removing females from the subjects’ territories during playback, logistical difficulties made this impossible; we could not capture females without

simultaneously capturing males. We controlled for variation in the subject's partner's song output during the Duetting Stage, as explained below.

Each playback trial consisted of 5 minutes of pre-playback silence, 2 minutes of neighbour male playback followed by 30 seconds of silence (i.e. the Intrusion Stage; 2.5 minutes in total), and 5 minutes of focal female playback (i.e. the subject's breeding partner) followed by 5 minutes of silence (i.e. the Duetting Stage; 10 minutes in total; Figure 2.1). We observed the behaviour of both the male subject and his breeding partner during the Intrusion and Duetting Stages. We did not conduct playback to neighbouring pairs on the same day, to avoid our playbacks having an influence outside of the territory of the subject. On a few rare occasions, neighbouring males approached the territory boundary and sang during our playback trials. We excluded these trials and re-conducted them during the following day.

The playback apparatus consisted of two camouflaged wireless loudspeakers (Scorpion TX200, FOXPRO Inc.) placed in vegetation 1m off the ground. We placed the loudspeaker for the Intrusion Stage 20-30m from the edge of the shared territory boundary with the closest neighbouring male. In a few cases when a male had no immediate neighbours, we placed the loudspeaker at the territory edge with the closest possible neighbouring male that the subject could conceivably hear. We placed the Duetting Stage loudspeaker (i.e. the loudspeaker playing the subject's partner's songs) near the centre of the subject's territory, and we always placed this second loudspeaker 50m away from the Intrusion Stage loudspeaker. Rufous-and-white Wren territories are large (300 – 1200 m²; Osmun and Mennill 2011), and therefore there were several trials where we placed the female speaker further away from the territory centre so

that it was always 50m away from the Intrusion Stage speaker. This ensured that subject males were always able to hear both speakers. We played all stimuli at 80 dB SPL, which we measured beforehand with a Casella CEL-24X sound level metre (C-weighting; fast response). We chose to play our stimuli at 80db because it reflects the typical amplitude of Rufous-and-white Wren songs at our study site, and has been used in previous playback studies with this species (e.g. Mennill 2006; Kovach et al. 2014).

During playback, an observer (ZAK) sat in a position concealed by vegetation, 15-20m away from the Duetting Stage loudspeaker, and recorded the experiment using a solid-state digital recorder (Marantz PMD661) and a shotgun microphone (Sennheiser MKH70) mounted to a small tripod. The observer was always positioned closer to the Duetting Stage loudspeaker in order to observe all vocal and physical behaviours of the subject male and his breeding partner during the experimental Duetting Stage (the Intrusion Stage was used as a priming treatment only). We placed flagging tape at 1m and 2m intervals in all 4 directions around the Duetting Stage speaker in order to help estimate the distance of the subject to the loudspeaker. The observer quietly dictated the identity and behaviours of focal birds into the microphone during playback, providing a time-synchronized record of the subject's vocalizations and physical activities.

Playback stimuli

We generated playback stimuli by isolating high-quality songs from recordings of birds previously collected at our study site. For neighbour male stimuli, we used recordings of male solo songs collected from the closest neighbouring individual for each focal pair. For focal

female stimuli, we used recordings of female solo songs collected from the subject's breeding partner. We created stimuli using Audition software (v 3.0; Adobe, San Jose, CA, U.S.A.). We selected one song with a high signal-to-noise ratio (assessed visually from the spectrogram), filtered out background noise from recordings with a 800 Hz high-pass filter, and standardized the song amplitude to -1dB so that all stimuli were broadcast at the same amplitude. We played songs in both treatments at a rate of 1 song every 10 seconds, consistent with the natural song rates of this species (Mennill and Vehrencamp 2005).

Playback response measurements

We used Syrinx PC (J. Burt, Seattle, WA, U.S.A.) to visualize audio recordings created during playback trials. We annotated these recordings to produce a time-stamped record of all duets, independent songs, and physical behaviours of subject birds. As in previous studies in Rufous-and-white Wrens, we defined a male-created duet as an instance where a male sang \leq 1.0 sec after the end of his female's song (either a playback song or a song from his actual partner; Mennill and Vehrencamp 2005). We defined an independent song as either a solo song (i.e. a song that was not preceded or followed by a partner's song by at least 1.0 sec) or a song that was sung as the first component of a duet (i.e. a song where the partner responded, creating a duet). From our annotations, we extracted three measurements of the subject's vocal behaviour, and four measurements of the subject's physical behaviour, during the Duetting Stage: (1) number of male-created duets in response to partner songs broadcast from the loudspeaker, (2) number of male-created duets in response to actual partner songs, (3) number of independent songs, (4) distance of closest approach to the female loudspeaker (m),

(5) latency to approach within 10m of the female loudspeaker (sec), (6) time spent within 10m of the female loudspeaker (sec), and (7) number of flights (i.e. the number of times a bird flew from one perch to another). From duetting variables (1) and (2), we created a single duet responsiveness variable, calculated as the total number of duets created with playback, divided by the total number of female songs the subject had an opportunity to perform a duet with (i.e. 30 songs from playback plus any additional independent songs uttered by the subject's partner). For birds that did not respond to playback, we assigned a distance of closest approach of 50m (we expect we would have detected any bird within this range) and a latency to approach within 10m of 600 seconds (i.e. the total length of the Duetting Stage).

Data analysis

We used duet responsiveness and number of male independent songs as our primary acoustic response measurements. To summarize variation in the four physical response measurements (measurements 4-7, above) we conducted a principal component analysis in JMP (v12.0; SAS Institute, Cary, NC). This analysis yielded one principal component (PC1) with an eigenvalue ≥ 1 (2.61), and this component explained 65.2% of the variation in the four original measurements. PC1 scores were positively associated with time spent within 10m of the female loudspeaker and number of flights, and negatively associated with distance of closest approach and latency to approach within 10m of the female loudspeaker. Thus, PC1 provided an indication of the physical response of the male, where higher scores indicated a higher intensity of physical response towards the female loudspeaker, consistent with physical mate guarding.

To assess differences in male vocal and physical behaviours across female fertility status, we conducted paired t-tests for our three key response variables. To investigate how male duetting behaviour changed across female fertility status, we used total duet responsiveness as our metric of duetting behaviour, and conducted paired t-tests on duet responsiveness between fertile and non-fertile stages. To investigate how independent song rates changes across female fertility status, we conducted paired t-tests on independent song rates between fertile and non-fertile stages. Finally, to investigate how male physical behaviour changed across female fertility status, we conducted paired t-tests on male physical response score (PC1) between fertile and non-fertile stages. All variables used in these analyses met assumptions of normality.

Given that we could not remove focal females from the subject's territory during playback trials, we wanted to investigate whether female behaviour during playback influenced the male subject's behaviour. To do this, we compared subject behaviour between trials where the females responded to playback (i.e. female approached within 50m of the Duetting Stage loudspeaker) and trials in which females did not respond (i.e. female remained >50m from the Duetting Stage loudspeaker); we used Wilcoxon Signed-rank tests due to the non-normality of our data. We defined a non-responsive female as one that did not approach the focal female loudspeaker (i.e. females remained at a distance >50 m throughout the Duetting Stage playback). All tests are two-tailed and all data are presented as means \pm standard errors.

We conducted a total of 42 playback experiments to the 21 playback subjects, each receiving playback at two breeding stages (i.e. fertile and non-fertile incubation). Males

responded to playback during the Duetting Stage in all trials by singing at least one song and approaching to within at least 20m of the female loudspeaker. Females responded in 29 out of 42 trials (69%). Of the 13 trials in which females did not respond, 2 were in the fertile period and 11 were in the non-fertile period.

Results

Male vocal responses to playback

The duetting behaviour of male Rufous-and-white Wrens varied significantly with female fertility status during the Duetting Stage of playback. Males answered a significantly higher proportion of female songs to create duets during the fertile period compared to the non-fertile period (fertile period: $44.0 \pm 5.0\%$ of female songs answered, non-fertile period: $33.0 \pm 5.0\%$; Paired t-test: $t_{20} = 3.1$, $p = 0.006$; Figure 2.2). Male duetting behaviour was not influenced by whether or not females responded to playback, because males answered a similar proportion of female songs to create duets in trials where their breeding partners responded compared to trials where they did not (female response: $38.9 \pm 3.9\%$; no female response: $37.7 \pm 8.0\%$; Wilcoxon signed-ranks test: $Z = 0.22$, $n = 42$, $p = 0.83$).

Males sang independent songs at similar rates during the Duetting Stage in the fertile period compared to the non-fertile period (fertile period: 30.6 ± 2.4 songs; non-fertile period: 27.5 ± 2.7 songs; Paired t-test: $t_{20} = 1.0$, $p = 0.45$; Figure 2.3). There was a significant propensity for males to sing more independent songs in trials where their females responded compared to trials where they did not (female response: 31.4 ± 2.2 songs, no female response: 23.7 ± 2.6 songs; Wilcoxon signed-ranks test: $Z = 1.97$, $n = 42$, $p = 0.049$).

Male physical responses to playback

The physical behaviour of male Rufous-and-white Wrens varied significantly with female fertility status during the Duetting Stage. Male physical response intensity scores (a principal component score, PC1, summarizing four physical response measurements) were significantly higher during the fertile period compared to the non-fertile period, indicating that males responded with greater physical intensity towards the loudspeaker when their females were fertile (Paired t-test: $t_{20} = 5.55$, $p < 0.0001$; Figure 2.4). Additionally, male physical response intensity scores were significantly higher in trials where their breeding partners responded to playback compared to trials where they did not (Wilcoxon signed-ranks Test: $Z = 2.67$, $n = 42$, $p = 0.008$; Figure 2.5).

Discussion

The playback responses of male Rufous-and-white Wrens support the Paternity Guarding Hypothesis. After experiencing an intrusion from a neighbouring male, subject males answered a significantly higher proportion of female songs to create duets during the fertile period compared to the non-fertile period, suggesting that duets serve a paternity guarding function in this species. In support of another one of our predictions, males exhibited a more intense physical response towards the loudspeaker simulating their breeding partners during the fertile period compared to the non-fertile period, suggesting that males also use physical mate guarding behaviours to protect their paternity. In contrast to our third prediction, males did not sing independent songs at a higher rate during the fertile period compared to the non-fertile period, suggesting that higher independent song rates do not function in acoustic

paternity guarding, and that the increased duet responsiveness of males was not a product of increased song output. Our study is among the first to provide experimental support for the idea that duets function as acoustic paternity guards in a duetting species, and our results indicate that male Rufous-and-white Wrens use both duets and physical behaviours to guard their females during the fertile period.

Acoustic paternity guarding

Male Rufous-and-white Wrens appeared to use vocal duets to guard their females during the fertile period. In contrast, many previous studies of seasonal variation in duetting behaviour show no evidence for higher duetting rates during the female fertile period (e.g. Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2009; Dowling and Webster 2013). Very few studies, however, have used playback to experimentally simulate an aggressive context where paternity guarding would be expected to occur, particularly where males are given an opportunity to answer many of their partner's songs. Rufous-and-white Wrens are unusual among duetting species that have been studied to date in that males answer the highest proportion of female songs to create duets during the fertile period in natural contexts, and the results of our experiment corroborate this finding (Topp and Mennill 2008). Rufous-and-white Wrens are one of only three species which appear to use duets to acoustically guard females during the fertile period (Marshall-ball et al. 2006; Baldassare et al. 2016), highlighting the need for more experimental studies to test this hypothesis in diverse species.

There are several possible explanations for why an acoustic paternity guarding strategy could be advantageous for male Rufous-and-white Wrens. First, ecological conditions at our

study site may necessitate an acoustic signalling strategy in the context of paternity guarding. Birds in our study population live in dense forests with substantial visual obstruction from vegetation, where it is likely difficult for males to maintain visual contact with partners over longer distances. A previous study in this species found that birds use duets to find each other and maintain contact over long distances, lending support to the idea that maintaining visual contact with a partner may be difficult (Mennill and Vehrencamp 2008). As such, males may need to use duets to advertise their own location and the paired status of their partner to intruders during the fertile period to prevent extra-pair mating attempts. Alternatively, males may signal their commitment to their breeding partner by creating duets with them, thereby dissuading them from engaging in extra-pair mating or divorce behaviours (Hall 2004). Female Rufous-and-white Wrens initiate the majority of divorce events and exhibit higher breeding dispersal than males, suggesting that females actively make decisions about mate choice during the breeding season (Graham et al. in press; Mennill unpublished data). Therefore, it may be critical for males to sing duets with their females during the fertile period to ensure mate retention and the ability to breed throughout the wet season.

The low rates of extra-pair offspring in some tropical species have been attributed to several different ecological factors (reviewed in Macedo et al. 2008), including reduced breeding synchrony as a result of longer and more unpredictable breeding seasons (e.g. Stutchbury and Morton 1995; Morton et al. 1998), low breeding densities resulting in fewer extra-pair mating opportunities (e.g. Verboven and Mateman 1997), higher annual adult survival (Mauck et al. 1998; Arndol and Owens 2002), increased male parental care dissuading females from engaging in extra-pair matings (Gowaty 1996; Morton et al. 1998; Albrecht et al.

2006), and long-term breeding partnerships (e.g. Chu et al. 2002). Interestingly, although Rufous-and-white Wrens exhibit low levels of extra-pair offspring in their nests (3% of offspring in 6% of broods; Douglas et al. 2012), only a few of these explanations apply to this species. For example, breeding synchrony is extremely high in our study population immediately after the first rainfall of the year (i.e. when we conducted the majority of playbacks), because pairs begin to build nests and lay eggs extremely rapidly during this time (Topp and Mennill 2008). In closely-related Buff-breasted Wrens, where breeding synchrony is very low, there were no extra-pair offspring across the population and males did not sing more duets with their females in the fertile period, indicating that low breeding synchrony may have contributed to higher female fidelity in this species (Gill et al. 2005). Additionally, although male Rufous-and-white Wrens help to build nests and provide care to their offspring, their level of investment appears be lower in comparison to other duetting species (i.e. they provide only 1/3 of nestling provisioning trips and do not incubate the eggs; Hall 1999; Gill et al. 2005) Finally, the relationship between breeding density and rates of extra-pair paternity in this species is unclear; population size, and probably breeding density, at our study site fluctuates greatly from year-to-year and appears to be directly related to local climate during the previous year (Woodworth et al. in prep). These characteristics, in addition to the results of our study, suggest that low rates of extra-pair paternity in Rufous-and-white Wrens are probably not due to ecological differences between tropical and temperate species, and instead that both acoustic and physical male paternity guarding strategies may be effective at limiting extra-pair mating opportunities.

Several studies have directly tested the Paternity Guarding Hypothesis by investigating how male duetting behaviour affects rates of extra-pair offspring in nests. In Crimson-breasted Shrikes, males that sang more duets with their females did not experience lower rates of extra-pair offspring in their nests, suggesting that duets were not effective paternity guards in this species (Van den Heuvel et al. 2014). In contrast, male Red-backed Fairy-wrens that exhibited stronger duetting responses during simulated intrusions had fewer extra-pair offspring in their nests, indicating that duets were effective at limiting extra-pair mating attempts from rival males. Interestingly, Red-backed Fairy-wrens have higher rates of extra-pair paternity in their nests (47% of offspring in 60% of broods; Baldassare et al. 2016) compared to Crimson-breasted Shrikes (20% of offspring in 32% of broods; Van den Heuvel et al. 2014), suggesting that there may be increased selection on acoustic paternity guarding strategies in species with lower female fidelity. Rufous-and-white Wrens exhibit low levels of extra-pair paternity in their nests, suggesting that there is minimal need for paternity guards, yet our results indicate that they exhibit pronounced paternity guarding behaviours (Kokko Morrell 2005; Douglas et al. 2012). This suggests that low rates of extra-pair paternity in Rufous-and-white Wrens may be a product of effective paternity guarding strategies. Ultimately, only the assessment of how duetting behaviour affects rates of extra-pair paternity across a wide-range of duetting species will help to elucidate the broad evolutionary pressures acting on these behaviours (Macedo et al. 2008).

We found that males did not sing more independent songs in the fertile period compared to the non-fertile period, indicating that males do not increase their overall song rates to guard their females and protect their paternity. This result suggests that the increase in

male duet responsiveness during the fertile period was a result of a change in song usage rather than simply a product of an overall increase in singing rate. Increased male song rates during the fertile period have been shown in males of some temperate species (e.g. Møller 1988; Bruni and Foote 2014; Sexton et al. 2007), although not in others (e.g. Gil et al. 1999; Turner and Barber 2004), providing evidence that this is not a widespread paternity guarding strategy across birds. In Rufous-and-white Wrens, singing more independent songs could be interpreted as a strategy to stimulate a response from a partner, and therefore advertise her mated status to intruders. Testing this idea is difficult, however, and this strategy is unlikely to be effective in cases where females are motivated to seek extra-pair mating opportunities and are therefore unlikely to be responsive to their partner's songs.

Physical paternity guarding

In support of one of our predictions, males exhibited a more intense physical response towards the focal female loudspeaker during the fertile period compared to the non-fertile period. This suggests that males may have attempted to physically guard their females during the fertile period, as they approached the focal female loudspeaker more quickly, more closely, and maintained close proximity for longer periods of time. Additionally, males exhibited a significantly greater physical response during trials when their females responded to playback, indicating that the presence of the actual breeding partner elicited a more intense physical response from subject males during playback. The strength of these trends provide strong support for the idea that physical mate guarding behaviours are important for male Rufous-and-white Wrens in protecting their paternity during the female fertile period.

Our results indicate that, in addition to acoustic duetting, physical mate guarding may be an effective strategy for male Rufous-and-white Wrens to protect their paternity. Rufous-and-white Wrens are similar to many other avian species which have been shown to physically guard their partners, both in temperate species (reviewed in Birkhead and Møller 1992; e.g. Westneat 1994; Currie et al. 1999; Komdeur et al. 1999; Evans et al. 2008; Foote et al. 2008), and in other duetting species (e.g. Hall and Magrath 2000; Hall and Peters 2009; Dowling and Webster 2017). Physical mate guarding has been shown to effectively reduce rates of extra-pair paternity in some species (e.g. Komdeur et al. 1999; Currie et al. 1999; Chuang Dobbs et al. 2001; Brylawski and Whittingham 2004), and could be one mechanism to explain the low levels of extra-pair paternity exhibited in Rufous-and-white Wrens (Douglas et al. 2012). Our results support previous observations in this species in which breeding partners appear to spend a lot of their time in close proximity to each other while foraging and nest-building during the early breeding season (Z. Kahn pers. obs). In tropical species living in dense habitats, physical behaviours could be extremely important to prevent extra-pair copulations, as acoustic guarding can only be effective when a female chooses to sing. The strong physical mate guarding behaviour displayed by males in our experiments, in addition to the evidence of duetting as an acoustic paternity guard, suggests that low rates of extra pair paternity in our population are likely a product of these paternity guarding strategies rather than by high female fidelity.

Due to the logistical difficulties in removing females from territories, we were unable to fully control for the effect of female behaviour on the responses of subject males. When we compared male physical response scores between trials where females did and didn't respond

to playback, we found that males responded with higher physical intensity in trials where their females were present. This suggests that physical responses of males and female were positively correlated, and that males exhibited a stronger physical response towards the female loudspeaker when their actual female partners were more responsive. There are two potential problems that arise from this: (1) Since there were many more unresponsive females during the non-fertile period (11 of 13 trials with unresponsive females occurred during the non-fertile period), our results could partially be a function of differences in female behaviour across breeding stages, and (2) Subject males may have perceived the focal female speaker as an intruding female rather than their breeding partner. Despite this, we are confident that the presence of focal females did not confound our results, for the following reasons: (1) Male subjects created duets with the female loudspeaker at similar rates in trials where females did and didn't respond to playback. This indicates that males continued to treat the focal female speaker as their partner even in the presence of their mate because, to the best of our knowledge, males do not create duets with birds other than their social partner (Mennill and Vehrencamp 2005; Mennill 2006; Mennill and Vehrencamp 2008; Kovach et al. 2014; Hick et al. 2015), (2) Similar playback protocols have been used successfully in three other species of duetting wrens, including two studies in Black-bellied Wrens in which females were not removed from territories (Logue 2007; Logue 2008; Templeton et al. 2013; Rivera-Cáceras et al. 2016), and (3) Subject males could be expected to respond with higher physical intensity during trials when their partners responded, as the presence of their females would provide them with additional signals to physically guard their partners. In addition, the thick vegetation at our study site would have made it difficult for males to maintain visual contact with their partners

at all times, suggesting that males would have a difficult time differentiating the focal female speaker from their partner. We therefore suggest that the physical behaviour of males during our experiments was primarily influenced by female fertility status, and that, although males appeared to more aggressively guard the focal female loudspeaker when their actual breeding partners were present during playback, this did not confound our results.

Conclusion

Using a playback experiment, we found evidence supporting the Paternity Guarding Hypothesis in Rufous-and-white Wrens; males exhibited differences in both physical and vocal behaviours across female fertility status. Males responded to a higher proportion of female songs to create duets in the female fertile period compared to the non-fertile period, suggesting that they were attempting to acoustically protect their paternity, but there were no differences in independent song rates between fertility periods. We also found a strong difference in male physical response intensity between fertility periods, because males appeared to physically guard their partners during the fertile period by approaching the focal female loudspeaker more quickly and closely compared to the non-fertile period. Our results suggest that Rufous-and-white Wrens employ a dual paternity guarding strategy, using both vocal and physical behaviours to guard their partners during the fertile period. This study is among the first to provide experimental support for the idea that vocal duets function as acoustic paternity guards, further highlighting the need to experimentally study duet function across a wide range of species and ecological contexts.

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Figures

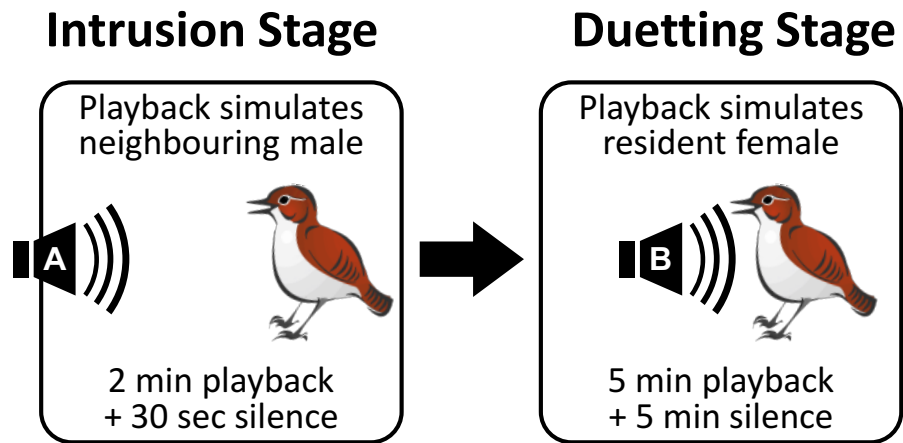


Figure 2.1: A visual timeline for the two-stage procedure of our playback experiment. Both stages were repeated for each subject when their partner was fertile, and when she was not fertile.

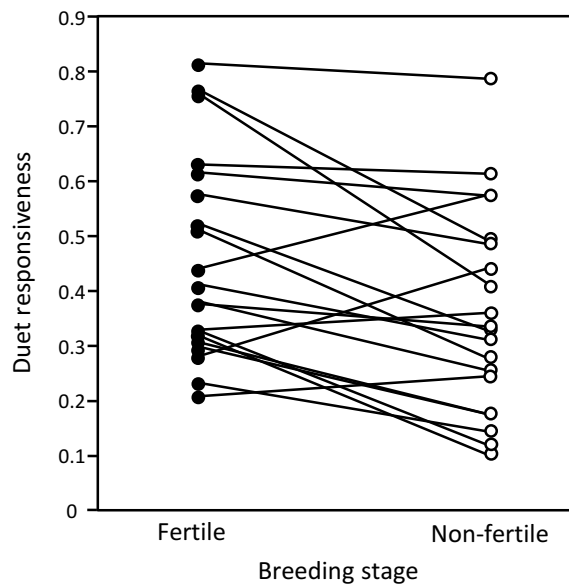


Figure 2.2: Male Rufous-and-white Wrens answered a significantly higher proportion of female songs (i.e. female songs from playback + actual female songs) in the fertile period (black circles) compared to the non-fertile period (white circles) during the Duetting Stage of the playback experiment. This graph shows paired comparisons of the duet responsiveness of individual males between the fertile and non-fertile breeding stages.

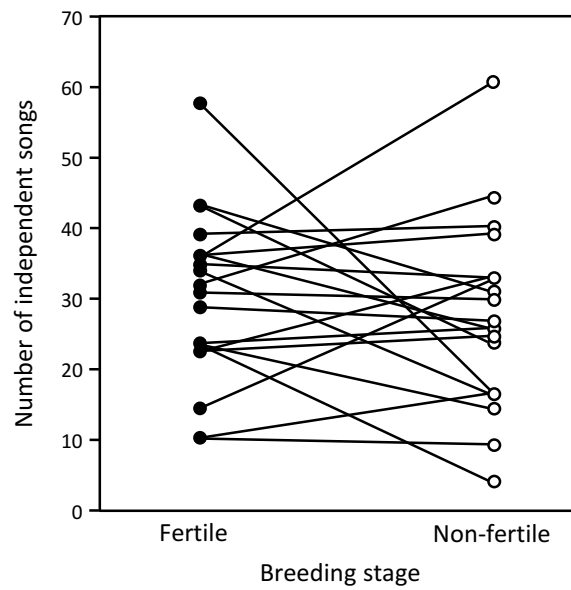


Figure 2.3: Male Rufous-and-white Wrens did not sing more independent songs in the fertile period (black circles) compared to the non-fertile period (white circles) during the Duetting Stage of the playback experiment. This graph shows paired comparisons of the number of independent songs by individual males between the fertile and non-fertile breeding stages.

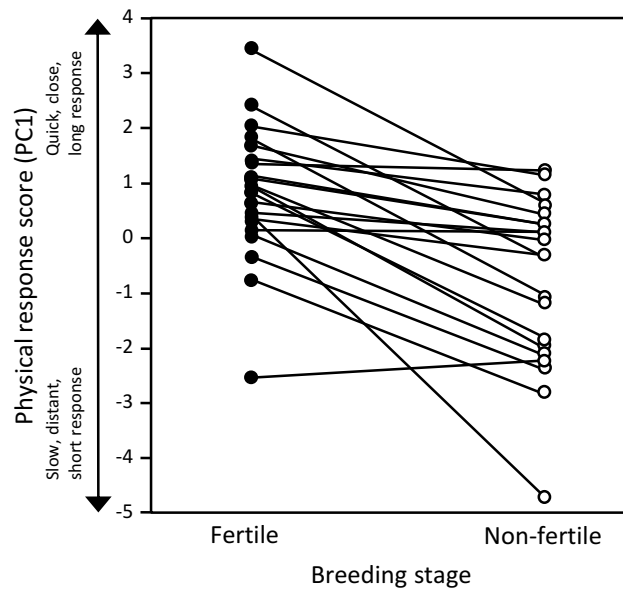


Figure 2.4: Male Rufous-and-white Wrens responded with significantly greater physical intensity towards the focal female loudspeaker in the fertile period (black circles) compared to the non-fertile period (white circles) during the Duetting Stage of the playback experiment. This suggests that males attempted to physically guard their females during this time. This graph shows paired comparisons of the physical intensity response (i.e. PC1 scores) of individual males between the fertile and non-fertile breeding stages.

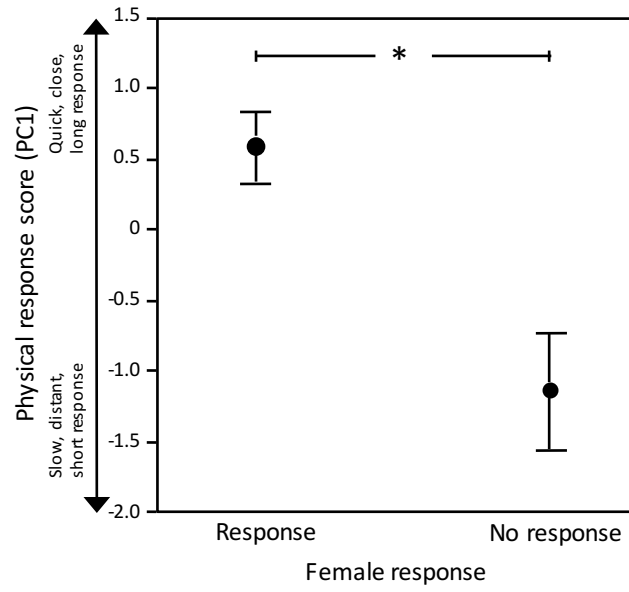


Figure 2.5: Male Rufous-and-white Wrens responded with a significantly greater physical intensity towards the focal female loudspeaker during trials when females responded to playback (n = 29) compared to trials when they did not (n = 13). Black circles represent means with standard error bars.

Chapter 3: Duetting behaviour does not signal future parental investment in Rufous-and-white Wrens

Chapter summary

In many tropical animals, breeding partners combine their vocalizations to create vocal duets. Although duets have been shown to function in territory defense, mate guarding, and maintaining contact, few studies have investigated the hypothesis that duetting behaviour can signal willingness or ability to invest effort into aspects of monogamous partnerships. In this study, we tested the hypothesis that duets are related to investment in future reproductive activities (i.e. the Signalling Commitment Hypothesis), by investigating the relationship between pre-breeding singing behaviour and subsequent parental investment during the nest-building and nestling-provisioning breeding stages. We recorded singing behaviour and reproductive activities from 38 breeding pairs in a colour-marked population of Rufous-and-white Wren (*Thryophilus rufalbus*) in Costa Rica. We did find support for predictions of the Signalling Commitment Hypothesis, because we found no relationships between the singing behaviour of an individual, or that of its breeding partner, and its own nest-building or nestling-provisioning effort, suggesting that duetting behaviour does not signal willingness or ability to provide future parental investment. Additionally, there was a significant positive correlation between the nest-building effort of breeding partners, suggesting that individuals adjusted their own level of parental investment by assessing their partner's behaviour around the nest during the nest-building stage. This study is the first to directly assess the relationship between singing behaviour and parental investment in a duetting species, and suggests that duets do not function as displays involved in post-pairing mate assessment or signalling parental commitment in Rufous-and-white Wrens.

Introduction

In many animals, individuals can evaluate the quality of potential breeding partners by assessing behavioural and ornamental traits, including acoustic signals (e.g. Buchanan and Catchpole 2000; Forsman and Hagman 2006; Vannoni and McElligott 2008), colour signals (e.g. Hill 1991; Baldauf et al. 2011, Smith et al. 2014), physical ornaments (e.g. Voltura et al. 2002; Pizzolon et al. 2012; Tibbetts et al. 2015), and courtship displays (e.g. Knapp and Kovach 1991; Green and Krebs 1995; Suzuki et al. 2013; Chou and Backwell 2016). These traits convey important information that can be used by individuals to assess and evaluate potential breeding partners, including fitness benefits that an individual may gain from pairing with the animal that possesses the trait. These fitness benefits include indirect benefits, such as increased viability of offspring via higher quality genes or enhanced attractiveness of offspring (Fisher 1930; Hamilton and Zuk 1982; Heywood 1989), as well as direct benefits, such as enhanced fertility and fecundity, access to higher quality ecological resources such as territories, nest sites, or food, or increased parental investment (Hoelzer 1989; Hamilton 1990; Andersson 1994).

Phenotypic traits that honestly indicate future parental investment have been especially well-studied in birds, probably because many birds exhibit elaborate ornamental traits and provide extensive parental care to their offspring (Cockburn 2006). For example, many studies in temperate species have shown relationships between parental investment (i.e. higher nestling-provisioning rates or more defensive displays at the nest towards predators) and visual traits, such as structural and carotenoid-based plumage brightness (e.g. Hill 1991; Linville et al. 1998; Casagrande et al. 2006; Silva et al. 2008), as well as acoustic traits, such as song rate (e.g. Greig-Smith 1982; Welling et al. 1997; Hofstad et al. 2002; Dolby et al. 2005) and

song complexity (e.g. Buchanan and Catchpole 2000; Bartsch et al. 2015). The majority of these studies, however, have been conducted in temperate species, where it is primarily females that assess male traits prior to mate selection at the beginning of the breeding season.

Remarkably few studies have investigated the importance of post-pairing mate assessment in tropical species, many of which have prolonged breeding seasons, defend territories year-round, and form long-term pair bonds, allowing individuals to evaluate the quality of their breeding partners over long time frames outside of the context of mate selection (Stutchbury and Morton 2001; Wachtmeister 2001). For instance, both males and females may assess traits that indicate the level of parental investment their partners will provide after pairing, thereby allowing individuals to adjust their own level of parental investment over the course of a prolonged breeding season, as well as make future decisions related to partnership divorce. Additionally, in many tropical species, both males and females exhibit highly ornamented traits, such as elaborate vocalizations and plumage colouration, yet few studies have assessed the importance of post-pairing traits in both male and female animals. For example, female song is an ancestral trait in songbirds, occurs in a diverse group of avian families, and is much more common than previously thought (Odom et al. 2014), highlighting the need to investigate the functions of acoustic post-pairing displays in avian species where both sexes sing.

In many animals, particularly in the tropics, mated pairs combine their vocalizations into coordinated vocal duets by overlapping their vocalizations or alternating their vocalizations in rapid succession (Farabaugh 1982; Hall 2004; Tobias et al. 2016). Once considered a rare behaviour, duetting is now known to occur across a wide range of animal taxa, including

primates (Geissman 2002; Caselli et al. 2015), anurans (Emerson and Boyd 1999), cetaceans (Lilly and Miller 1961), insects (Bailey 2003), bats (Carter et al. 2008), and many species of birds (Farabaugh 1982; Hall 2004). In birds, duetting is present across 49% of avian families and in as many as 16% of all bird species, primarily in the tropics (Tobias et al. 2016). Across species, there is evidence that duets function in joint territory defense (reviewed in Dahlin and Benedict 2014), acoustic mate guarding (Rogers et al. 2007; Tobias and Seddon 2009), and maintaining contact in dense habitats (Logue 2007; Mennill and Vehrencamp 2008).

One longstanding but poorly-studied hypothesis for the function of vocal duets is the Signalling Commitment Hypothesis, which states that the effort required to create coordinated duets with a breeding partner requires a learning period and is therefore a reflection of pair bond strength (Wickler 1980). An interesting alternative interpretation of this hypothesis is that duetting behaviour can signal commitment to a monogamous partnership, such as willingness or ability to invest effort into partnership aspects such as territory defense or parental investment (Hall 2004). Under the Signalling Commitment Hypothesis, an individual's duetting behaviour – for example, their propensity to answer a partner's song to create a duet – should provide an honest indication about the quality of the partner and the willingness or ability to invest effort to future reproduction (Hall 2004). Duetting is common among socially monogamous bird species with year-round territoriality and long term pair bonds, and is an acoustic post-pairing display that occurs throughout the year in many species (e.g. Topp and Mennill 2008; Benedict 2010; Tobias et al. 2016). As such, duetting birds represent a special opportunity to investigate the importance of elaborate post-pairing displays that could function in mate assessment and signalling commitment to long-term partnerships, potentially by

providing an indication of future parental investment. No study to date has directly assessed the relationship between singing behaviour and future parental investment in a duetting species, and whether or not duetting behaviour is a post-pairing display involved in mate assessment by both males and females, as has been shown for male song in temperate species (e.g. Greig-Smith 1982; Buchanan and Catchpole 2000).

In this study, we tested the Signalling Commitment Hypothesis in a tropical duetting songbird: the Rufous-and-white Wren (*Thryophilus rufalbus*). We quantified three aspects of singing behaviour during the pre-breeding season: (1) the number of duets that a bird created (i.e. the number of songs they sang in response to their partner, turning their partner's song into a duet), (2) the proportion of partner songs answered to create a duet, and (3) the number of independent songs (i.e. songs sung as solos, or the first part of duets before a partner responded). We then quantified two aspects of parental behaviour during the nest-building and nestling-provisioning stages of the breeding season: (1) nest trip rate and (2) relative parental effort. We tested the Signalling Commitment Hypothesis by assessing (1) the relationship between an individual's singing behaviour and its own level of parental investment, and (2) the relationship between a partner's singing behaviour and the individual's level of parental investment. Duet rates and the proportion of partner songs answered to create duets (i.e. duet responsiveness) have been suggested to be important indicators of partnership commitment in duetting species (Hall 2004). Similarly, male song rates have been shown to correlate with future parental investment in some temperate songbird species (e.g. Greig-Smith 1982; Welling et al. 1997), and could function similarly in both male and female Rufous-and-white Wrens. In accordance with the Signalling Commitment Hypothesis, we predicted that there would be a

positive relationship between an individual's singing behaviour in the pre-breeding season and its own parental investment, whereby birds that sang more duets with their partner in the pre-breeding season would signal their commitment to providing greater parental investment during the nest-building and nestling-provisioning breeding stages. Consequently, we predicted that there would be a positive relationship between an individual's parental investment and the singing behaviour of its partner, whereby individuals with partners that sang more songs and created more duets with them would elicit greater parental investment from that individual. Additionally, we assessed the relationships between male and female parental investment within pairs during nest-building and nestling-provisioning, and predicted that nest-building and nestling-provisioning rates would be correlated between breeding partners.

Methods

Study species and general field methods

We studied a colour-banded population of Rufous-and-white Wrens during five years between 2009 and 2016 (2009-2010, 2012-2013, and 2016). This study is part of a long-term investigation of this population, located in Sector Santa Rosa of the Area de Conservación Guanacaste in northwestern Costa Rica (10°51'N, 85°36'W). Each year, we captured birds using mist nets and gave each individual a unique combination of three coloured bands and one numbered aluminum band to facilitate identification in the field. Across the five years of the investigation, we monitored a total of 95 birds (46 males and 49 females) from 57 unique pairs. We were unable to sample each of these pairs during both breeding stages (15/57 pairs were observed during both breeding stages), but we sampled the singing behaviour and parental

investment during at least one breeding stage for each pair. We observed 38 pairs during the nest-building stage and 35 pairs during the nestling-provisioning stage, and 13 birds were observed as a member of a partnership with different partners in different years. Rufous-and-white Wrens are socially monogamous, duetting songbirds that form long-term pair bonds and defend territories year-round (Mennill and Vehrencamp 2005; Douglas et al. 2012). Both males and females answer each other's songs to create duets, and both sexes contribute to parental activities by helping to build nests and provision offspring, although only females incubate the eggs and brood the young (Mennill and Vehrencamp 2005; Topp and Mennill 2008). We studied birds each year from early-April to late-June, a time that coincides with the end of the dry pre-breeding season and the beginning of the wet breeding season (i.e. after the first large rainfall of the year; Topp and Mennill 2008). For all territorial pairs, we defined social breeding partners as birds that sang and duetted with each other on a nesting territory prior to breeding, and together built nests and provisioned offspring during the breeding season.

Acoustic recordings and sound analysis

We obtained recordings of each pair in our study during the pre-breeding season before nest-building and nestling-provisioning had begun (typically from early-April until mid-May). We collected a total of 327 hours of recordings (average of 5.5 ± 1.8 recording hours per pair). We recorded 47 out of 57 pairs (82%) during at least two recording sessions, and we recorded every pair for a minimum of 1 hour. We collected most recordings during 1-2 hour focal recording sessions during the dawn chorus, wherein a recordist followed pairs around their territories and dictated the identity of the singer into the recorder after each song (sexes were differentiated using unique colour-band combinations and frequency differences between male and female

songs; Mennill and Vehrencamp 2005). We collected these recordings using a directional shotgun microphone (Sennheiser ME66/ME67/MKH70) attached to a solid-state digital recorder (Marantz PMD-660/PMD-661/PMD-670). For several pairs, we supplemented our focal recordings using automated digital recorders (Wildlife Acoustics Song Meter SM2, or, in 2009, custom-made autonomous recorders made up of Marantz PMD660 recorders and Sennheiser ME62 microphones; details in Mennill 2014). For these recordings, we were confident in the identity of each singer because we placed the recorder in the centre of the territory, ensuring we only obtained songs from the focal pair. We collected all recordings used for analysis in this study between 0500 and 0900h, a time that coincides with the period of highest singing activity for Rufous-and-white Wrens at our study site (Mennill and Vehrencamp 2005).

We analyzed the singing behaviour of focal pairs by visualizing spectrograms of our recordings using Syrinx-PC sound analysis software (John Burt, Seattle, WA). We categorized vocalizations based on the sex of the bird producing each vocalization, and whether it was a solo song or a song that was part of a duet. Following Mennill and Vehrencamp (2005), we classified a song as part of a duet if it overlapped a partner's song, or was sung ≤ 1.0 s after a partner's song. We classified each duet as either male-created (i.e. the male sang the second song, thereby turning the female's song into a duet) or female-created (i.e. the female sang the second song, thereby turning the male's song into a duet). We classified a song as a solo if it was sung independently from the partner (i.e. greater than 1.0s before or after a partner's song). We then calculated three acoustic measurements: (1) independent song rate (the number of songs sung independently of a bird's partner, i.e. solo songs plus duets where the bird sang the first component, divided by the total number of recording hours); (2) duet rate

(the number of duets created by the bird, divided by the total number of recording hours); and (3) duet responsiveness (the proportion of independent songs sung by a bird's partner which an individual sang in response to create a duet). As in Topp and Mennill (2008), we calculated male duet responsiveness as the number of male-created duets divided by independent female songs, and female duet responsiveness as the number of female-created duets divided by the number of independent male songs.

Quantifying parental investment

To measure the parental investment of focal birds, we conducted detailed observations of birds at their nests during both the nest-building stage (n = 41 watches on 38 pairs) and nestling-provisioning stage (n = 58 watches on 35 pairs). During these observation sessions, an observer sat in a concealed position 15-20m away from the nest and quietly recorded the identity of each bird when it made a trip to the nest with nesting material or food. We observed 1175 nest trips during nest-building watches and 595 nest trips during nestling-provisioning watches for a total of 1770 trips across both breeding stages. We defined a nest trip as an instance where a bird went completely inside of the nest (or early nest structure) with nesting material or food. On rare occasions, we were unable to differentiate between the male and the female as they entered the nest, due to the high density of obstructive vegetation at our study site. These observations accounted for 4.5% of all trip observations (3.7% of nest-building trips and 6.1% of nestling-provisioning observations); we excluded these observations from our analyses. Within each year, we observed each pair during both breeding stages when possible (n = 15; 26% of all pairs), but were unable to do so for most pairs due to high nest predation rates at our study site (Douglas et al. 2012). There were several instances where a male arrived

at the nest with food but the female was inside the nest brooding the nestlings, or where birds did not bring new building material to the nest but went inside to improve the structure; we included these events with the other nest trips.

During the nest-building stage, we conducted a minimum of one 60-minute nest watch for each pair, with some watches spanning 90 or 120 minutes (average of 68.1 ± 2.7 minutes per watch and 73.4 ± 4.2 watch minutes per pair). We conducted at least one nest watch for every pair, and conducted watches on multiple days for several pairs (3/38 pairs). Birds in our study population build conspicuous domed nests primarily in bullhorn acacia trees (*Vachellia collinsii*) 2-10 m off the ground; these trees are relatively scarce in the more mature evergreen sections of the study site where Rufous-and-white Wrens breed, making them easy to locate during nest-building. During the nestling-provisioning stage, we conducted a minimum of one 60-minute nest watch for each pair (average of 72.4 ± 3.9 minutes per watch and 93.5 ± 9.9 watch minutes per pair), and conducted multiple watches for 14 of 35 pairs (40%). Due to the fact that the timing of these watches was not standardized across years, we later divided nestling-provisioning nest-watches into three provisioning periods: “early nestling-provisioning” watches were those when nestlings were 1-4 days old, “middle nestling-provisioning” watches were those when nestlings were 5-9 days old, and “late nestling-provisioning” watches were those when nestlings were 10+ days old (nestlings appear to fledge around day 16; D. Mennill pers. obs.). We determined the ages of nestlings by periodically checking inside nests and assessing nestling features throughout the breeding season, or, for nests that were too difficult to reach, by forward-dating from our best estimate of clutch initiation date. We conducted a minimum of one 60-minute nest watch for all pairs during at least one provisioning period,

across two periods for 14 pairs (40%), and across all three periods for 5 pairs (14%). In total, we conducted 18 nest watches on 17 pairs during the early provisioning period (67.5 ± 10.2 minutes per watch and 71.5 ± 14.8 watch minutes per pair), 24 nest watches on 22 pairs during the middle provisioning period (73.7 ± 15.7 minutes per watch and 80.4 ± 23.5 watch minutes per pair), and 16 watches on 13 pairs during the late provisioning period (75.8 ± 6.8 minutes per watch and 91.8 ± 21.4 watch minutes per pair).

From our field observations, we calculated two measures of parental investment: (1) nest trip rate (i.e. the number of trips to the nest with building material or food made by the bird divided by the number of nest watch hours), and (2) relative parental effort (i.e. the proportion of trips made to the nest made by a bird relative to the total number of trips made by the pair). We then created four variables to be used for analyses for both males and females: (1) nest-building trip rate, (2) relative nest-building effort, (3) nestling-provisioning trip rate, and (4) relative nestling-provisioning effort.

Statistical analysis

To analyze differences in nest trip rates between sexes across breeding stages and provisioning periods, and the relationship between an individual's nest trip rate and that of its breeding partner, we created Linear Mixed Models (LMM) using the lme4 package (Bates et al. 2015) in R (version 3.2.3, R Core Team, 2016). For both breeding stages, we created a model with number of trips to the nest as the response variable. In both models, we assigned sex (two levels: male and female), provisioning period (three levels: early, middle, and late), and partner nest trip rate as fixed predictor variables, and included pair id as a random effect to account for

repeated sampling of pairs. We did not control for the effect of brood size in these models because (1) we were unable to determine the brood size for 9 pairs in our study due to extremely high nests, (2) there was relatively little variation in brood size among the pairs we sampled (85% of pairs had either 3 or 4 nestlings), and (3) including brood size in the models did not change our results, but did reduce the overall power of the model due to the reduction in sample size.

To analyze differences in relative parental effort between sexes across breeding stages and nestling-provisioning periods, we used two-tailed T-tests in JMP (v12.0; SAS Institute, Cary, NC). First, within the nest-building stage, we tested for differences in relative building effort between the sexes. Second, within the nestling-provisioning stage, we tested for differences in relative provisioning effort between sexes, and within each sex across nestling-provisioning periods. We assessed the distribution of all response variables prior to analyses to ensure that assumptions of normality were met.

To analyze the relationship between singing behaviour and parental investment, we created Generalized Linear Mixed Models (GLMM) using the lme4 package (Bates et al. 2015) in R (version 3.2.3, R Core Team, 2016). This allowed us to account for non-normality in our response variables, and for a non-balanced design in our data (i.e. because we were unable to sample all pairs across all breeding stages and provisioning periods; Bolker et al. 2008). We created four models in each breeding stage (i.e. nest building and nestling-provisioning) for a total of eight models, and we used a Laplace approximation method. Within each breeding stage, we created two models with number of trips to the nest and relative parental effort as

the continuous response variables. For our trip rate models, we used a Poisson error distribution, and included the number of watch hours as an offset argument to convert this variable into a rate. For our relative parental effort models, we used a Binomial error distribution, and included the total number of trips as a weight argument. In all models, we assigned independent song rate, partner independent song rate, duet rate, and partner duet rate as continuous predictors, and assigned individual and pair identification as random effects to account for variation in individual behaviour and repeated sampling of pairs. We did not include duet responsiveness as a predictor variable in our models because (1) some females did not sing at all, meaning that their male breeding partners had no chance to create duets with them and thus our sample size would be reduced, and (2) duet rate and duet responsiveness were highly correlated, meaning that we could include duet rate in our models as a proxy for duet responsiveness.

Results

Parental investment

Male and female Rufous-and-white Wrens contributed nearly equal effort to building nests. Parents of each sex made trips to the nest with building material at similar rates (males: 13.2 ± 1.6 trips per hour, females: 11.6 ± 1.3 trips per hour; LMM: estimate = 0.16 ± 0.51 , $t = 0.32$, $p = 0.75$) and, proportionately, contributed similar relative nest-building effort (Figure 1; T-test: $t_{80} = 0.86$, $p = 0.39$). There was a significant positive correlation between the nest-building trip rates of breeding partners (Figure 3; LMM: estimate = 0.88 ± 0.05 , $t = 16.6$, $p < 0.0001$). There was considerable variation among males and females in both nest-building trip

rates (males: coefficient of variation (CV) = 75.8%; females: CV = 68.7%) and relative nest-building effort (males: CV = 51.1%; females: CV = 54.3%), and this variability was similar between sexes.

In contrast to nest-building, parental investment during the nestling-provisioning stage was heavily female-biased. Females provisioned nestlings at significantly higher rates than males overall (males: 2.98 ± 0.78 trips per hour, coefficient of variation (CV) = 129.8%; females: 4.69 ± 0.79 trips per hour, CV = 65.7%; LMM: estimate = -2.15 ± 0.57 , $t = 4.01$, $p = 0.0001$), and in all three provisioning periods (early provisioning: Figure 2; estimate = 2.15 ± 0.54 , $t = 4.01$, $p = 0.001$; middle provisioning: Figure 2; estimate = 2.15 ± 0.54 , $t = 4.01$, $p = 0.001$; late provisioning: Figure 2; estimate = 2.15 ± 0.54 , $t = 4.01$, $p = 0.001$). The low values and high variation in male nestling-provisioning rates was driven in-part by several males that did not provision nestlings at all during our watches (10 out of 35 males, 28%). We did not find a significant effect of provisioning period on nestling-provisioning trip rates (middle period: estimate = 0.02 ± 0.60 , $t = 0.03$, $p = 0.98$; late period: estimate = 1.22 ± 0.68 , $t = 1.80$, $p = 0.07$), but there was a non-significant tendency for both sexes to provision nestlings at higher rates during the late provisioning period (Figure 2). There was a non-significant positive correlation between the nestling-provisioning rates of breeding partners (Figure 4; LMM: estimate = 0.16 ± 0.09 , $t = 1.74$, $p = 0.08$).

Similarly, females contributed significantly greater relative nestling-provisioning effort than males overall (Figure 1; males: CV = 81.1%; females: CV = 42.9%; T-test: $t_{34} = 3.70$, $p =$

0.0008), and during all three provisioning periods (Figure 1; early: T-test: $t_{34} = 3.70$, $df = 34$, $p = 0.0008$; middle: T-test: $t_{46} = 3.01$, $p = 0.004$; late: T-test: $t_{30} = 4.94$, $p < 0.0001$).

Singing versus parental investment during nest-building

We found no significant relationships between singing behaviour in the pre-breeding season and parental investment during the nest-building stage for either sex (Table 1). For males, independent song rate (GLMM: estimate = -0.004 ± 0.004 , $z = -0.99$, $p = 0.32$), partner independent song rate (estimate = 0.032 ± 0.04 , $z = 0.82$, $p = 0.41$), duet rate (estimate = 0.04 ± 0.21 , $z = 0.16$, $p = 0.87$), and partner duet rate (estimate = 0.04 ± 0.08 , $z = 0.44$, $p = 0.66$) were not significant predictors of male nest-building rate (Table 1). Similarly, independent song rate (GLMM: estimate = -0.005 ± 0.01 , $z = -0.87$, $p = 0.38$), partner independent song rate, (estimate = 0.02 ± 0.05 , $z = 0.38$, $p = 0.70$), duet rate (estimate = -0.21 ± 0.26 , $z = -0.82$, $p = 0.42$), and partner duet rate (estimate = 0.05 ± 0.10 , $z = 0.48$, $p = 0.63$) were not significant predictors of relative nest-building effort (Table 1). Females followed an identical pattern to males; Independent song rate (GLMM: estimate = 0.02 ± 0.03 , $z = 0.59$, $p = 0.56$, $n = 41$ observations on 38 females), partner independent song rate (estimate = 0.001 ± 0.004 , $z = 0.41$, $p = 0.69$), duet rate (estimate = 0.04 ± 0.08 , $z = 0.49$, $p = 0.49$), and partner duet rate (estimate = 0.21 ± 0.16 , $z = 1.34$, $p = 0.18$) were not significant predictors of female nest-building trip rate (Table 2). Similarly, independent song rate (estimate = -0.03 ± 0.05 , $z = -0.66$, $p = 0.51$), partner independent song rate (estimate = 0.003 ± 0.01 , $z = 0.59$, $p = 0.56$), duet rate (estimate = -0.06 ± 0.12 , $z = -0.48$, $p = 0.63$), and partner duet rate (estimate = 0.36 ± 0.33 , $z = 1.10$, $p = 0.27$) were not significant predictors of relative nest-building effort (Table 2).

Singing versus parental investment during nestling-provisioning

For males, we found no relationships between singing behaviour or partner singing behaviour and parental investment during the nestling-provisioning stage. Independent song rate (estimate = -0.005 ± 0.006 , $z = -0.88$, $p = 0.38$), partner independent song rate (estimate = -0.06 ± 0.05 , $z = -1.30$, $p = 0.20$) duet rate (estimate = 0.18 ± 0.18 , $z = 0.99$, $p = 0.32$) and partner duet rate (estimate = -0.12 ± 0.09 , $z = -1.39$, $p = 0.16$) were not significant predictors of male nestling-provisioning trip rate (Table 1). Similarly, independent song rate (GLMM: estimate = -0.012 ± 0.007 , $z = -1.85$, $p = 0.06$), partner independent song rate (estimate = -0.08 ± 0.05 , $z = -1.46$, $p = 0.14$), duet rate (estimate = 0.27 ± 0.22 , $z = 1.26$, $p = 0.21$), and partner duet rate (estimate = -0.12 ± 0.10 , $z = -1.24$, $p = 0.21$) were not significant predictors of male relative nestling-provisioning effort (Table 1). Similarly, in females, independent song rate (estimate = 0.01 ± 0.02 , $z = 0.71$, $p = 0.48$), partner independent song rate (0.003 ± 0.003 , $z = 1.13$, $p = 0.26$), duet rate (-0.03 ± 0.03 , $z = -0.89$, $p = 0.37$), and partner duet rate (estimate = -0.02 ± 0.08 , $z = -0.30$, $p = 0.77$) were not significant predictors of female nestling-provisioning trip rate (Table 2). Similarly, independent song rate (estimate = 0.03 ± 0.04 , $z = 0.74$, $p = 0.46$), partner independent song rate (estimate = 0.01 ± 0.007 , $z = 1.39$, $p = 0.17$), duet rate (0.08 ± 0.10 , $z = 0.78$, $p = 0.44$), and partner duet rate (-0.06 ± 0.19 , $z = -0.31$, $p = 0.76$) were not significant predictors of female relative nestling-provisioning effort (Table 2).

Discussion

We found no support for the Signalling Commitment Hypothesis in Rufous-and-white Wrens. Five years of data revealed that, in both males and females, duetting behaviour does

not predict subsequent parental investment during the breeding season. During both the nest-building and nestling-provisioning stages, birds that created more duets with their breeding partner did not bring building material or food to the nest at higher rates, and did not increase their relative parental effort by carrying out a higher proportion of trips to the nest.

Consequently, we found no relationships between parental investment and partner singing behaviour, as birds did not adjust their own level of parental investment in response to the singing behaviour of their partners. However, we found that nest-building trip rates were positively correlated between the sexes, indicating that birds may use other signals to assess the parental quality of their breeding partners or increase their nest-building effort in response to their partner's behaviour. Overall, our results suggest that Rufous-and-white Wren do not use duets to signal their willingness or ability to provide parental investment, and therefore that duets likely do not function as post-pairing displays of parental commitment in this species.

Duetting behaviour and signalling parental investment

To our knowledge, this study is among the first to investigate how duetting behaviour relates to future parental investment in a monogamous bird species, and the first to do so in a wild population. A recent study of captive Zebra Finches (*Taeniopygia guttata*) found that structural components of nest-site call duets contained information about future incubation bout length, where duets were used to negotiate incubation investment between pair members after foraging trips (Boucaud et al. 2015). However, no study to date has investigated how duetting behaviour relates to parental investment (i.e. nest building and nestling-provisioning) in a natural context. Here, we found that Rufous-and-white Wrens duets do not

signal willingness or ability to provide future parental investment, and so do not appear to function as signals of pair bond strength in the context of parental commitment.

Although our results are consistent with the idea that duets do not signal parental quality in Rufous-and-white Wrens, an alternative explanation is that other aspects of duetting behaviour that we did not measure were better indicators of parental commitment in this species. In his proposal of the Signalling Commitment Hypothesis, Wickler (1980) suggested that duets should require a learning period, such that the time required to achieve coordinated duets with a partner provides a mechanism to strengthen the pair bond and dissuade individuals from leaving their partners. In Rufous-and-white Wrens, simply answering a partner's song to form duets appears to be relatively easy to achieve, because birds sing loosely-coordinated polyphonal duets, and our observations suggest that new pairs are able to sing duets with no obvious learning period involved (Mennill and Vehrencamp 2005; Mann et al. 2009). Therefore, other aspects of duetting behaviour that we did not measure in this study, such as the precision in answering a partner's songs or adherence to a set of answering rules (i.e. a duet code), could require greater effort to achieve and therefore provide a better indication of an individual's parental commitment (Hall 2004; Rivera-Cáceras et al. 2016). For example, individuals that are faster to answer their partner's songs may signal the length of their partnership and therefore their commitment, because producing coordinated duets requires a learning period in some species and may also be indicative of being attentive to a breeding partner (Hall 2004; Hall and Magrath 2007; Logue 2007). Similarly, some duetting species, including two wren species closely related to Rufous-and-white Wrens, adhere to a "duet-code", a strict set of answering rules whereby individuals consistently answer different

partner songs with certain song types to form consistent duet combinations (e.g. Logue 2006; Mann et al. 2009; Rivera-Càceras et al. 2016). In species which exhibit duet codes, correctly answering a partner's song may serve as a signal of partnership commitment, especially if this ability requires time to learn (Marshall-ball et al. 2006; Templeton et al. 2013). In Canebrake Wrens (*Cantorchilus zeledoni*), both duet precision and adhering to a duet code requires a learning period, such that birds singing precisely timed duets that closely follow the correct answering rules with a partner signal longer and stronger partnerships (Rivera-Càceras et al. 2016). Preliminary work from our lab suggests that Rufous-and-white Wrens may also adhere to a duet code (Osmun 2011), and future work in this species should investigate whether duet code adherence or duet precision can be related to aspects of partnership commitment such as parental investment.

A second explanation for our results is that behavioural traits unrelated to duetting serve as better indicators of parental investment in Rufous-and-white Wrens. During both the nest-building and nestling-provisioning stages, nest trip rates were positively correlated between breeding partners. This indicates that Rufous-and-white Wrens may use other phenotypic traits to assess the parental quality of their partners, or that individuals adjust their own levels of parental investment by assessing the parental behaviour of their partners around the nest. If this latter interpretation is correct, our results indicate that birds may evaluate the parental quality of their partners by observing their behaviour around the nest during the breeding season, and may use this information to adjust their own level of investment. Our results are similar to those found in Buff-breasted Wrens (*Thryothorus leucotis*), a closely-related species of duetting wren wherein both nest-building and provisioning-trip rates were

significantly correlated between breeding partners (Gill and Stutchbury 2005). In this species, the rate at which males built secondary roosting nests was positively related to nestling-provisioning rates and female survival, suggesting that females could gain fitness benefits by assessing male nest-building behaviour (Gill and Stutchbury 2005). In Rufous-and-white Wrens, males also build secondary breeding nests that can be used for subsequent breeding attempts (although they do not appear to initiate this type of nest until after the first breeding attempt is underway), suggesting that similar nest-building displays could occur in this species. We were unable to assess similar relationships in our study due to the low number of pairs for which we were able to collect both nest-building and nestling-provisioning data for, but preliminary analysis reveals that similar mechanisms could occur in Rufous-and-white Wrens. Although many acoustic and physical traits have been shown to accurately predict the parental quality of males in many temperate species (e.g. Greig-Smith 1982), many more studies are needed in tropical duetting species to assess the importance of post-pairing traits in long-term mate assessment, such as duetting and nest-building displays (Wachtmeister 2001; Gill and Stutchbury 2005).

A final explanation for our results is that duets may have signalled willingness or ability to invest effort into aspects of parental investment that we did not investigate in this study. We assumed that there should be selection in Rufous-and-white Wrens for increased parental effort during nestling-provisioning (i.e. higher feeding rates), because this would result in greater offspring survival and therefore higher reproductive success (e.g. Eggert et al. 1998; Gubernick and Teferi 2000; Møller 2000). However, in areas with high nest-predation rates such as the tropics, parents often reduce the number of feeding trips they make to the nest in order

to minimize predation risk from visual predators (Martin et al. 2000; Eggers et al. 2005; Massaro et al. 2008; Martin et al. 2011). Therefore, selection for higher nestling feeding rates may not be as strong for birds at our tropical study site, where the threat of nest predation from mammalian and avian predators is extremely high (Douglas et al. 2012). Instead, birds experiencing high levels of nest predation may compensate for reduced feeding rates by increasing the quantity and quality of food items brought to the nest during each feeding trip (Martin et al. 2000), something we were unable to quantify in our study. Additionally, we did not assess post-fledging juvenile care, which occurs for longer periods in tropical species and is important to the survival of offspring (Russell et al. 2004; Tarwater and Brown 2010). In Rufous-and-white Wrens, juveniles continue to be cared for by their parents for at least 6-8 weeks after fledging (Ahumada 2001), and this is likely a critical period for young birds to ensure survival during their first year (particularly during their first dry season when resources are limited).

Our results suggest that in Rufous-and-white Wrens, simply answering a partner's song to create duets primarily serves functions unrelated to post-pairing mate assessment and signalling future parental investment. Unlike many temperate species, in which positive associations between singing behaviour and parental investment have been found (e.g. Greig-Smith 1982; Buchanan and Catchpole 2000), Rufous-and-white Wrens are tropical songbirds that hold territories year-round and have prolonged breeding seasons (Ahumada 2001). As such, there is greater importance for them to ensure access to ecological resources throughout the year, particularly during the dry season when ecological resources are limited (Ahumada et al. 2001; Stutchbury and Morton 2001). Year-round territoriality appears to have been important for the evolution of avian duets, and many studies have demonstrated the role of

duetting in territory defense behaviours (Dahlin and Benedict 2014; Logue and Hall 2014; Tobias et al. 2016). In Rufous-and-white Wrens, several previous studies have provided evidence to indicate that territory defense is an important function of vocal duets, where both males and females sing duets at high rates during the pre-breeding period when food resources are limited, increase the number of duets they sing in response to simulated duetting intruders, and sing duets in response to intruders regardless of their sex or paired status (Mennill 2006; Mennill and Vehrencamp 2008; Topp and Mennill 2008). Duets also appear to serve other important communication functions in this species, such as to maintain contact in dense habitat and coordinate breeding activities (Topp and Mennill 2008; Mennill and Vehrencamp 2008). Although previous authors have suggested that duets are multi-functional signals (Mennill and Vehrencamp 2008; Benedict 2010; Dahlin and Benedict 2014), revealing commitment to future parental investment does not appear to be one of those functions in Rufous-and-white Wrens.

Conclusion

Our results do not support the Signalling Commitment Hypothesis for the function of acoustic duets in Rufous-and-white Wrens, as we did not find a positive association between duetting behaviour and subsequent parental investment in males or females. In both sexes, birds that sang more independent songs (i.e. initiated more duets) and created more duets with their partner did not contribute more effort to nest-building or nestling-provisioning, and birds did not adjust their own levels of parental investment in response to the singing behaviour of their partners. These results suggest that Rufous-and-white Wrens cannot assess the duet responsiveness of their breeding partner to evaluate their parental quality, and therefore do not adjust their own level of parental investment in response to these acoustic signals. This

study is the first to directly investigate the relationship between singing behaviour and future parental investment in a duetting species, and among one of the only studies to assess the importance of an acoustic post-pairing display for mate assessment in both males and females. Our work suggests that duetting behaviour is potentially unimportant for post-pairing mate assessment in Rufous-and-white Wrens, and therefore provides new insight into the functions of acoustic duets across species.

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Tables

Table 3.1: There were no significant relationships between individual or partner singing behaviour during the pre-breeding season and subsequent parental investment during the breeding season for male Rufous-and-white Wrens.

Male Parental Investment Models (GLMM)			
	Estimate \pm SE	z value	p value
Nest-building Trip Rate			
Duet Rate	0.04 \pm 0.21	0.16	0.87
Partner Duet Rate	0.04 \pm 0.08	0.44	0.66
Independent Song Rate	-0.004 \pm 0.004	0.99	0.32
Partner Independent Song Rate	0.03 \pm 0.04	0.82	0.41
Nest-building Effort			
Duet Rate	-0.21 \pm 0.26	-0.82	0.42
Partner Duet Rate	0.05 \pm 0.10	0.48	0.63
Independent Song Rate	-0.01 \pm 0.01	-0.87	0.38
Partner Independent Song Rate	0.02 \pm 0.05	0.38	0.70
Nestling-provisioning Trip Rate			
Duet Rate	0.18 \pm 0.18	0.99	0.32
Partner Duet Rate	-0.12 \pm 0.09	-1.39	0.16
Independent Song Rate	-0.01 \pm 0.01	-0.88	0.38
Partner Independent Song Rate	0.06 \pm 0.05	-1.30	0.20
Nestling-provisioning Effort			
Duet Rate	0.27 \pm 0.22	1.26	0.21
Partner Duet Rate	-0.12 \pm 0.10	-1.24	0.21
Independent Song Rate	0.01 \pm 0.01	-1.85	0.06
Partner Independent Song Rate	-0.08 \pm 0.05	1.46	0.14

Table 3.2: There were no significant relationships between individual or partner singing behaviour during the pre-breeding season and subsequent parental investment during the breeding season for female Rufous-and-white Wrens.

Female Parental Investment Models (GLMM)			
	Estimate \pm SE	z value	p value
Nest-building Trip Rate			
Duet Rate	0.04 \pm 0.08	0.49	0.49
Partner Duet Rate	0.21 \pm 0.16	1.34	0.18
Independent Song Rate	0.02 \pm 0.03	0.59	0.56
Partner Independent Song Rate	0.001 \pm 0.004	0.41	0.69
Nest-building Effort			
Duet Rate	-0.06 \pm 0.12	-0.48	0.63
Partner Duet Rate	0.36 \pm 0.33	1.10	0.27
Independent Song Rate	-0.03 \pm 0.05	-0.66	0.51
Partner Independent Song Rate	0.003 \pm 0.01	0.59	0.56
Nestling-provisioning Trip Rate			
Duet Rate	-0.03 \pm 0.03	-0.89	0.37
Partner Duet Rate	0.02 \pm 0.08	-0.30	0.77
Independent Song Rate	0.01 \pm 0.02	0.71	0.48
Partner Independent Song Rate	0.003 \pm 0.003	1.13	0.26
Nestling-provisioning Effort			
Duet Rate	0.08 \pm 0.10	0.78	0.44
Partner Duet Rate	-0.06 \pm 0.19	-0.31	0.76
Independent Song Rate	0.03 \pm 0.04	0.74	0.46
Partner Independent Song Rate	0.01 \pm 0.01	1.39	0.17

Figures

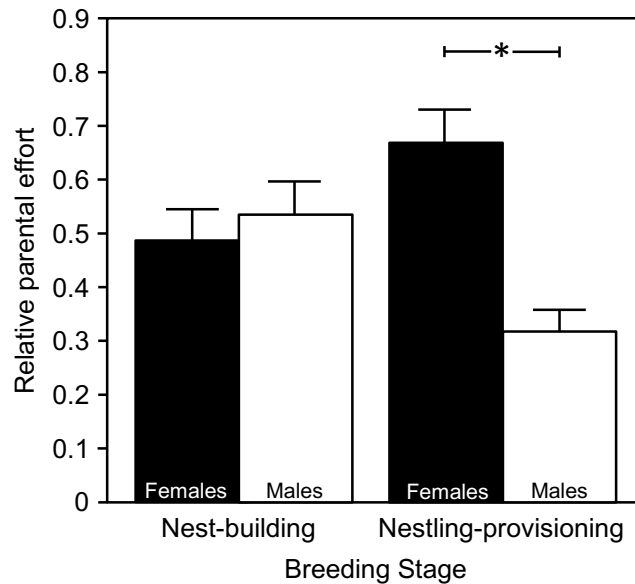


Figure 3.1: Female (black bars) and male (white bars) Rufous-and-white Wrens contributed similar parental effort during Nest-building (i.e. made a similar proportion of total trips to the nest with building material; $n = 38$ pairs), but females contributed significantly more effort than males during the nestling-provisioning stage (i.e. made a greater proportion of total trips to the nest with food; $n = 35$ pairs). Values for the nestling-provisioning stage are pooled from all three provisioning periods for each pair. Bars on the graph represent means with standard errors.

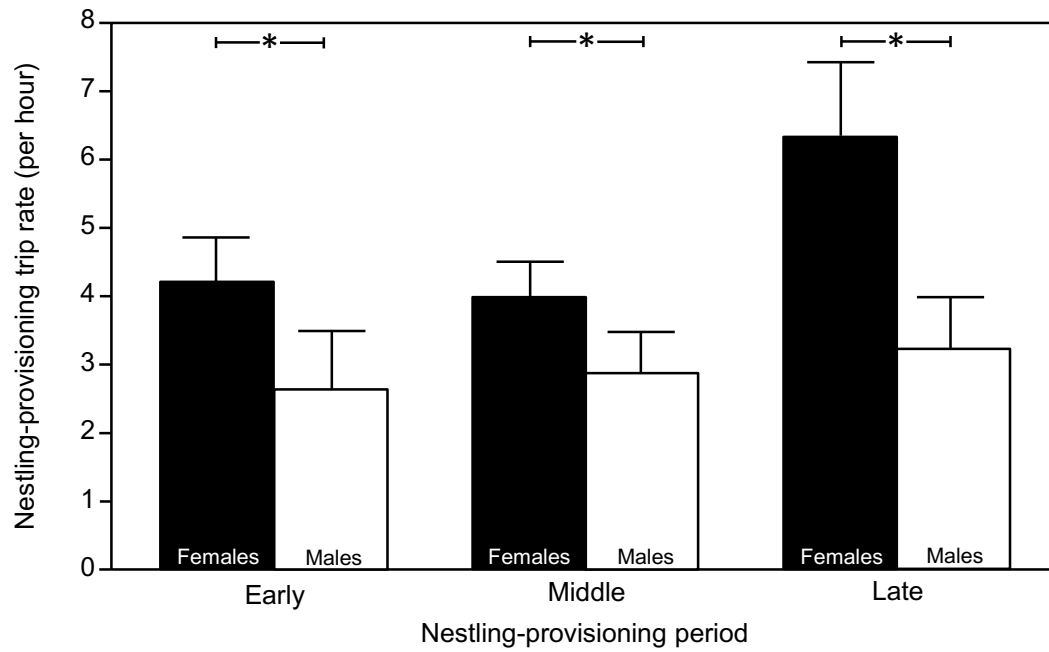


Figure 3.2: Female Rufous-and-white Wrens (black bars) provisioned nestlings at significantly higher rates than males (white bars) during the early (nestling age: 1-4 days; $n = 17$), middle (nestling age: 5-9 days; $n = 22$), and late (nestling age: 9+ days; $n = 13$) provisioning periods. Bars on the graph represent means with standard errors.

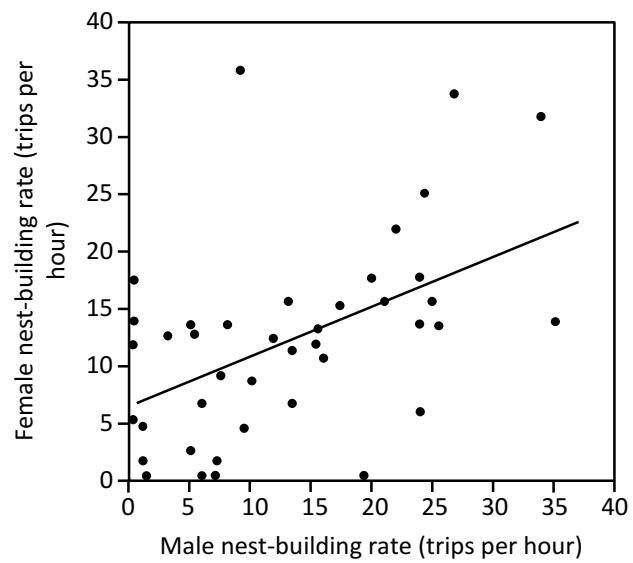


Figure 3.3: There was a significant positive correlation between the nest-building trip rates of male and female Rufous-and-white Wrens during the nest-building stage (n = 38).

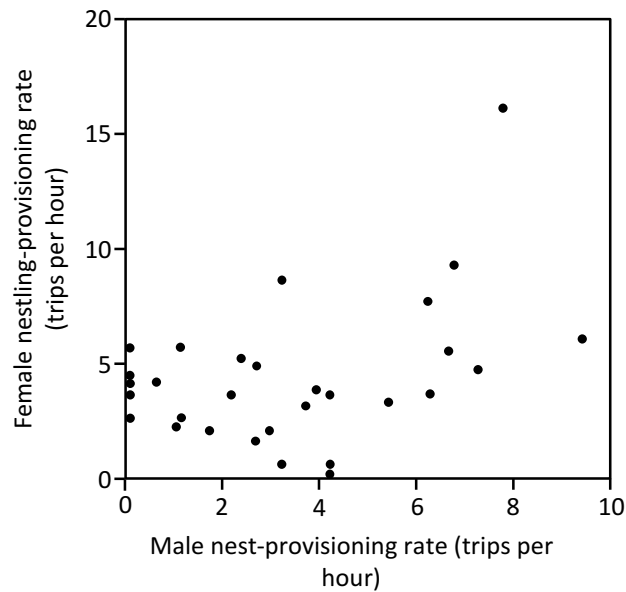


Figure 3.4: There was a non-significant positive correlation between the nestling-provisioning trip rates of male and female Rufous-and-white Wrens during the nestling-provisioning stage. Points represent nestling-provisioning trip rates for each pair that have been pooled from all provisioning periods ($n = 35$).

Chapter 4: General Discussion

In this thesis, I explored the evolution and ecology of vocal duetting behaviour in Rufous-and-white Wrens (*Thryophilus rufalbus*). In chapter 2, I investigated the hypothesis that duets are used by males to acoustically protect their paternity (i.e. the Paternity Guarding Hypothesis) using a novel experimental approach. I first played songs of a neighbouring male from the territory edge to simulate a rival seeking extra-pair copulations, and then played songs of the subject's breeding partner from the territory centre, giving the subject male an opportunity to create duets with his female. I conducted this experiment to males during both the female fertile period and the non-fertile incubation period. In support of the Paternity Guarding Hypothesis, male Rufous-and-white Wrens answered a higher proportion of female songs during the fertile period compared to the non-fertile period, suggesting that duets function as acoustic paternity guards. However, male independent song rates did not vary between fertility periods, indicating that the change in duet responsiveness arose due to a change in song use rather than an increase in overall song rate. Additionally, males displayed a significantly higher physical response intensity (i.e. they approached the female speaker quicker, closer, and for longer) during the fertile period, suggesting that physical behaviours were also important for paternity guarding. The results from this chapter suggest that there is intra-sexual conflict in Rufous-and-white Wrens during the breeding season, and that males use duets as well as physical behaviours to guard their females and protect their paternity during the fertile period.

In chapter 3, I explored the hypothesis that duets function as signals of partnership commitment (i.e. the Signalling Commitment Hypothesis) by investigating the relationship between pre-breeding duetting behaviour and future parental investment during the breeding

season. I analyzed singing and parental behaviour from 38 pairs of Rufous-and-white Wrens between 2009 and 2016, and then assessed how individual and partner singing behaviour was related to parental investment during nest-building and nestling-provisioning. In contrast to predictions of the Signalling Commitment Hypothesis, I found no relationships between individual or partner duetting behaviour and parental investment during either breeding stage. These results suggest that duet responsiveness does not signal future parental investment in Rufous-and-white Wrens, and therefore may not be a signal of partnership strength. However, I did find that nest-building and nestling-provisioning trip rates were positively correlated between breeding partners, suggesting that birds adjusted their own level of parental investment in response to the parental activities of their partner. The results from this chapter suggest that duets are not used by Rufous-and-white Wrens to assess the parental quality of their partners, and so do not function as a pair strengthening mechanism in this way.

The results from these two chapters further support the idea that the duets of Rufous-and-white Wrens are multi-functional signals (Mennill 2006; Mennill and Vehrencamp 2008; Topp and Mennill 2008), and that their functions can vary with ecological context. In chapter 2, I showed that duets play an additional role in acoustic mate guarding during the fertile period. This result provides clear evidence that Rufous-and-white Wrens duets are important for males in conflict-based contexts between breeding partners during the fertile period, in addition to being important in other cooperative contexts (i.e. territory defense and maintaining contact with breeding partners; Mennill and Vehrencamp 2008). Conversely, in chapter 3, I demonstrated that duets are not used by individuals to signal future parental investment, and therefore that duet responsiveness does not reflect the strength of a partnership in this way.

This result provides a compelling test of a poorly-studied hypothesis for duet function, and suggests that there is weak selection for duets to function as post-pairing signals of parental quality during the pre-breeding season. Together, these two chapters provide new insight into the functions of vocal duets across animals by demonstrating a clear function, and non-function, of this behaviour in Rufous-and-white Wrens across widely different ecological contexts.

An interesting avenue for future research in relation to chapter 2 would be to directly determine the relationship between duetting behaviour and extra-pair offspring. I was unable to assess this relationship due to a reduced population size during my field research, but this analysis is also complicated by high predation rates that are commonplace in the tropics, and the low rates of extra-pair offspring in Rufous-and-white Wrens (3% of offspring in 6% of broods; Douglas et al. 2012). However, a comparison of duet rates and parentage will provide a direct test of the Paternity Guarding Hypothesis, as it directly relates duetting behaviour with an important component of fitness (i.e. paternity lost to other males). For example, in Red-backed Fairy-wrens, males that created more duets with their females during playback suffered fewer extra-pair offspring in their nests, suggesting that duets act as acoustic paternity guards and can effectively reduce extra-pair mating opportunities (Baldassare et al. 2016). Future studies in Rufous-and-white Wrens should assess similar relationships over longer time frames, which would help to determine if the low rates of extra-pair paternity exhibited in this species can truly be attributed to acoustic or physical paternity guarding. Furthermore, many more studies are needed to determine the genetic mating systems of other duetting species in order to determine the role of duetting in acoustic paternity guarding and the broad ecological

selection pressures acting on extra-pair mating behaviour in duetting species specifically, and tropical species more broadly (Macedo et al. 2008).

An interesting avenue for future research in relation to chapter 3 would be to assess the importance of alternative aspects of duetting behaviour in the context of signalling partnership commitment. In chapter 3, I showed that duet responsiveness – the propensity for an individual bird to answer its partner’s song to form a duet – was not related to future parental investment. However, other aspects of duetting behaviour, such as duet precision (Hall and Magrath 2007; Rivera-Cáceras et al. 2016) or adherence to a duet code (Logue 2006; Templeton et al. 2013; Rivera-Cáceras et al. 2016), could provide a better indication of partnership commitment and coalition strength. For example, performing precisely coordinated duets with a partner signals coalition strength in Magpie-Larks, as more experienced pairs produce more coordinated duets in response to simulated intruders compared to new pairs (Hall and Magrath 2007). Similarly, the ability of Canebrake Wrens to produce precisely coordinated duets and adhere to a duet code increases over time, suggesting that these aspects of duetting behaviour honestly signal partnership length and commitment (Rivera-Cáceras et al. 2016). Future studies should address similar questions in Rufous-and-white Wrens by investigating whether this species requires a learning period to perform coordinated duets, and whether duet coordination or appropriately answering a partner’s song (i.e. adhering to a duet code) can be related to aspects of partnership commitment, such as parental investment (e.g. preliminary work from our lab suggests that Rufous-and-white Wrens may have a set of answering rules for creating duets; Osmun 2011). Furthermore, many more studies are needed to describe patterns of parental investment in duetting species, and if there are species in which duetting behaviour

signals investment in parental activities during nest-building and nestling-provisioning, as well as during post-fledgling care.

When considering the functions of vocal duetting behaviour, it is important to recognize that males and females may have different motivations for answering their partner's songs to create duets, and that these motivations may change across different ecological and social contexts (Logue and Krupp 2016; Odom et al. 2017). For example, one of the primary functions of duets in both male and female Rufous-and-white Wrens appears to be to defend ecological resources while defending year-round territories, because both males and females actively participate in duet creation in response to all types of territorial intrusions (Mennill 2006; Mennill and Vehrencamp 2008). This makes Rufous-and-white Wrens similar to many other duetting species in which territory defence has been shown to be the main function of vocal duets, and supports recent work suggesting that year-round territoriality is an important driver for the evolution of duetting behaviour in birds (reviewed in Dahlin and Benedict 2014; Tobias et al. 2016). In addition, both males and females move closer to each other after duetting, indicating that maintaining acoustic contact is another important function of duetting (Mennill and Vehrencamp 2008). In both cases, males and females appear to be equally motivated to answer their partner's songs, since they both can gain from ensuring access to ecological resources and knowing the location of their partner. In contrast, the results of this thesis indicate that duets are not always cooperative signals in this species, and that duetting may be co-opted to perform different functions in different contexts. For example, it is clear from chapter 2 that males have increased motivation to create duets with their females during the fertile period. In doing so, they may prevent their breeding partners from engaging in extra-pair

mating opportunities, ward off rival males seeking extra-pair copulations, dissuade their breeding partners from divorcing them, or stimulate reproductive physiology. Conversely, I demonstrated in chapter 3 that there is not selection on males and females to signal their parental investment through their duetting behaviour. Therefore, whether or not there are differences for males and females to use duets to signal partnership commitment or strengthen long-term pair bonds is still unclear in this species. It is important for future duetting researchers to recognize that duets often serve both cooperative and conflict-based functions, and to study this behaviour from the individual perspective of males and females separately (Dahlin and Benedict 2014; Logue and Krupp 2016).

In summary, my research provides new insight into the ecology and evolution of vocal duetting behaviour in tropical animals by testing two poorly-studied hypotheses for duet function in novel ways. I first demonstrated that male Rufous-and-white Wrens use duets to acoustically guard their females during the fertile period, and then that duets are not used by individuals to assess the parental investment of their mates and thereby signal commitment to a monogamous partnership. Vocal duetting is a remarkable behaviour which is associated with animals living at tropical latitudes. In these species, there are pronounced differences in ecology and natural history in comparison to temperate zone animals, including prolonged breeding seasons, long-term monogamous partnerships, year-round territoriality, convergent sex roles, and densely forested habitats (Stutchbury and Morton 2001; Stutchbury and Morton 2008). These ecological differences almost certainly contribute to the differences in acoustic signalling strategies between tropical and temperate animals, and are crucial to understanding the function of vocal duetting behaviour, because they provide the basis for differing selection

pressures acting on both males and females to produce these coordinated signals. The results of my thesis suggest that there is variation in the functions and importance of vocal duets across different ecological contexts. My thesis also highlights the importance of studying acoustic communication in tropical animals in which both males and females produce vocalizations, an area that has received considerably less empirical attention than in temperate species where acoustic signalling is heavily male-biased.

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